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803. *For* Steel, J. H. *read* Steele, J. H.

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Page 23. Five lines from bottom. *Delete* Utgitt av.
111. Eight lines from bottom. *For* Voorst *read* Koorst.
167. Table V. 179, Column 1. 180, Column 1. For *swammerdammi* read *swammerdami*.
168. For *Siphonocoetes* read *Siphonoecetes*.
175. Table XIV. Column 1. For *Trichobranchius* read *Trichobranchus*.
178. Appendix. Column 2. For *Trichobranchius* read *Trichobranchus*.
179. Column 1, line 18. For *Perioculoides* read *Perioculodes*.
179. Column 1, line 25. For *Meara* read *Maera*.
180. Column 1, at top. For *Actinea* read *Actinia*.
180. Column 1, 13 lines from bottom. For *rubicata* read *rubricata*.
180. Column 1, 9 lines from bottom. For *augusta* read *angusta*.

No. 3. Instructions for the preparation of manuscripts, column 1, 9th line from bottom. *For* 22 *read* 12.

Page 611. Line 3. *Delete* pituitary.
621. Text-fig. 4A. There should be no 'white-concentrating principle' at minus one.
658. Line 4. For *Geranomya* read *Geranomyia*.
663. Eight lines from bottom. For *Chaetoceras* read *Chaetoceros*.

DUNLOP
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Geo P Bidder.

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GEORGE PARKER BIDDER

1863–1953

The death of George Parker Bidder in his 91st year on 31 December 1953 has removed our last personal link with the days of the early development of the Marine Biological Association and its struggle for existence between the end of last century and a few years before the First World War. After the death of E. J. Allen in 1942 the only other survivor who had played a major part in the story besides Bidder was Walter Garstang, who died in 1949. Apart from those like Allen and Garstang, who were directly responsible for carrying out the work for which the Association was founded, no one contributed more vitally to its final success than Bidder, whose shrewd counsel and wise benefactions were undoubted factors in the survival of the Plymouth laboratory. In these days of recognition by the Government of the needs of science it is not easy for us to appreciate what the difficulties must have been for those who had to prove to an unresponsive audience that marine biological research was worth while. Those men were remarkable in their generation, and one of the most remarkable of them was G. P. Bidder.

George Parker Bidder was born in London on 21 May 1863. His life fulfilled the expectations aroused by the endowment of unusual gifts and characteristics passed to him through his parents. His father, George Parker Bidder, Q.C., was the son of George Parker Bidder, C.E., the 'calculating boy'. His mother was Anna McClean; her father was the engineer J. R. McClean, F.R.S., and her brother was Frank McClean, F.R.S. From his father's side George Parker Bidder the third inherited, among many gifts, especially his power of calculation and his unusual memory; from his mother's side came in part, at least, that remarkable vision and judgement which led him to do so many things at the right time, and his ability to handle people with ease and harmony.

His grandfather, George Parker Bidder the first, was born at Moretonhampstead on the north-eastern borders of Dartmoor in 1806. The son of a stone-mason, he had an amazing natural ability for calculation, and was taken round the countryside for profit as the 'calculating boy'. Thanks to the intervention of certain gentlemen he was enabled to have University training and eventually became a leading railway engineer, collaborating with the Stephensons. In later years he owned an estate at Mitcham, south of London, known as Ravensbury Manor, where his grandson often stayed as a child. Later, when

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owing to ill-health he moved to Ravensbury, Dartmouth in Devon, his son occupied Ravensbury Manor, Mitcham, and it became the grandson's home.

George Parker Bidder, the third, was educated at King's Preparatory School, Brighton, and at Harrow, where he was in the sixth under Dr Butler. While at Harrow he won the Prize Poem in 1881. On leaving Harrow Bidder spent one year at University College, London, on the advice of F. M. Balfour, attending the lectures of Ray Lankester, virtually the founder of the Marine Biological Association, its first Honorary Secretary, and its President from 1890 to 1929. After completion of this year he went as an Exhibitioner in Mathematics and Science to Trinity College, Cambridge, where he took the Natural Science Tripos in 1884 and 1886. On his arrival in Cambridge, Balfour having recently died, the lecturers in zoology were W. F. R. Weldon and S. F. Harmer. On one occasion these two took young Bidder shore-collecting in Guernsey and it is possible that this strengthened an interest in marine biological research apparently first aroused by reading Jules Verne's *Twenty Thousand Leagues Under the Sea*; although as a boy he had not been without experience of the sea, having cruised from Dartmouth in his grandfather's yacht *Mayfly*. He was later to own his own yachts the *Chanceit* and *Myrtle* in which he used to sail at Plymouth.

On the completion of his degree Bidder went to the Zoological Station at Naples where he held the Cambridge University table in 1887, '88 and '89, and was the guest of Anton Dohrn in 1890, '91 and '93. It was during these years that he did much of his experimental work on sponges which was not to see publication until some thirty years had passed.

While in Naples Bidder, whose appreciation of visual beauty was always acute, became much interested in sculpture and archaeology; this interest led later to a small publication entitled *Arcus*, giving convincing arguments that this word was used for bow-drills. During this period, also, he purchased a hotel in Naples which he called 'Parkers' and ran successfully for many years, an act which has now become legendary.

Bidder also became extremely proficient in long-distance and underwater swimming; he would no doubt have been fascinated by the possibilities for underwater research opened up by modern free-diving appliances.

Bidder made his first visit to the Plymouth laboratory in 1890, but he did not work there until 1893, being recorded in the Reports of Council for that and the succeeding two years as working on 'sponges'. In 1893 E. J. Bles was Director and E. J. Allen was a visiting research worker. It was then that Allen and Bidder met for the first time, and it was no doubt an appreciation of Allen's greatness, coupled with the deep friendship that developed between them, that moved Bidder to devote so much loving thought to the affairs of the Association. Allen became Director on 12 January 1894. One of his first publications after taking up his duties was a report on the sponge fishery of Florida and the artificial culture of sponges, prepared at the request of the

Colonial Office, with a view to the introduction of sponge culture in the Bahamas. Bidder was by then an authority on sponges, and it was natural that Allen should have called on him for assistance. Allen's report was followed in the *Journal* by a note by Bidder on projects for the improvement of sponge fisheries. Perusal of this paper shows how far ahead of his time Bidder was in his functional outlook. A reminder of his experience as an underwater swimmer also is to be found in his suggestion that naked divers should be encouraged to try the use of water spectacles: 'while every student knows that the imperfection of the submerged human eye can be corrected by convex lenses, there is a wide gulf of ignorance separating the student from the pearl-diver of the Indian Ocean'.

From 1893 to 1896 Bidder was a regular visitor at Plymouth, but in 1896 his father died and a great amount of business fell on his shoulders. Amongst other things he inherited interests in a dock, a dry dock, a colliery, a Danish gas company, a derelict Cornish lead mine, and a farm at Mitcham. He is not recorded among the list of visiting research workers at Plymouth again until the year 1899 when he married Marion Greenwood, a physiologist of some distinction, and they came to live in Plymouth. The Bidders remained in Plymouth until 1902 after the birth of their elder daughter Caroline, now Mrs Barclay Russell and the mother of two girls and a boy of whom their grandfather was very proud. They then moved to Cambridge and shortly into Cavendish Corner, which was to remain the home until he died. In 1903 was born their younger daughter Anna, who is now a Founder of the Association.

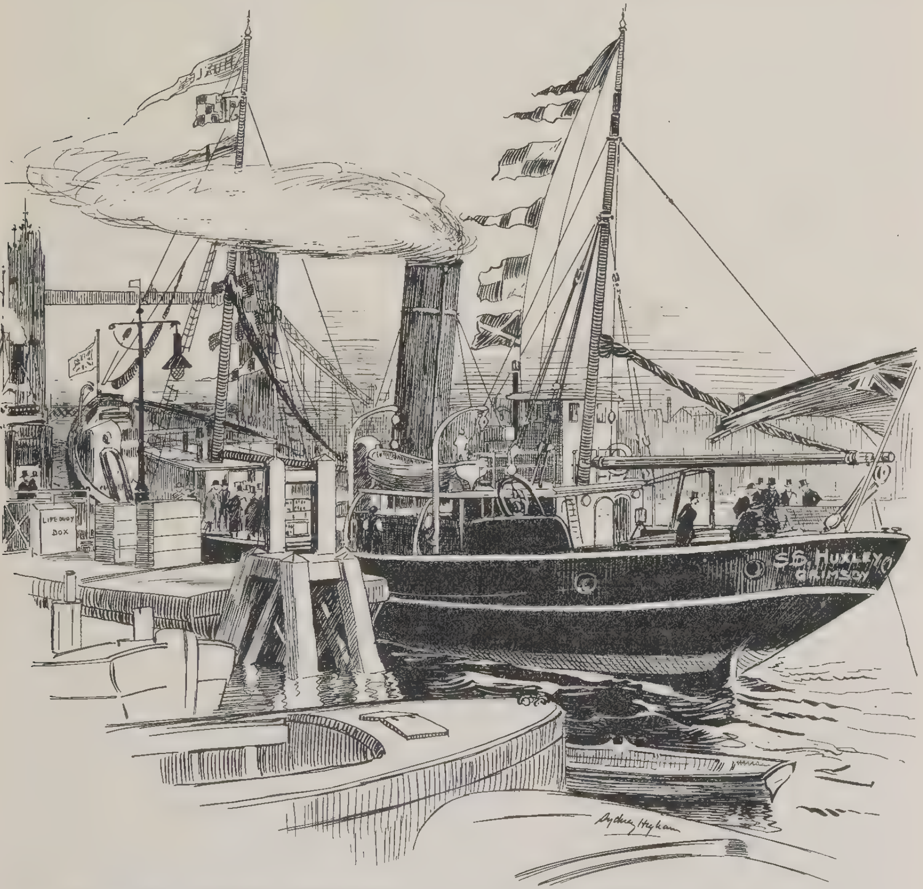
During the period since his father's death, in addition to his zoological and other cultural activities, Bidder had been attending monthly meetings of the Board of Directors of Cannock Chase Colliery (he was managing director from 1897 to 1908, and chairman from 1915 to 1919), making occasional visits to Denmark, and going annually to his hotel in Naples. In 1903 he began to complain of a tiredness from which he had been suffering for many years and by 1905 he was definitely shown to have tuberculosis. This illness, during which he was forbidden to use a microscope, struck at a time of life when he should have been in his prime. He made winter visits to Davos and to Assouan, and indeed was at one time given only a few months to live. Yet after 10 or 12 years of this semi-invalidism and care he made a complete recovery.

During those years, however, Bidder was in fact anything but inactive. He had the time to do an immense amount of wide reading, thus gaining that great breadth of knowledge from which he so evidently drew in his conversation and publications. For several years, from 1909 to 1913, he went with his family each autumn to Mundesley on the Norfolk Coast. Here he became very much interested in the local geology and coastal erosion. At home also he was known to spend much time in his bathroom experimenting with his bottom-trailers designed for the study of currents in the North Sea. The knowledge so derived led to a demand by the Admiralty for his services during the 1914–18 war when he was attached to H.M.S. *Vernon* for research. By 1909 he had already adopted those habits of unusual hours for which he became so well known and which persisted until the end of his life; in fact he was often said to be like the Snark in that 'he would frequently breakfast with afternoon tea and dine on the following day'.

It was during the earlier period of his illness that Bidder was also especially active in the affairs of the Marine Biological Association. By 1909 he had played a part which must have been vital to the future of all marine biological and fisheries research in this country. In 1902, under the auspices of the International Council for the Exploration of the Sea, the southern North Sea and English Channel were allotted to England for investigation. There being no government department in England concerned with Fishery Research, H.M. Treasury requested that the Association should act as agents to the Government for the equipment and working of a vessel to do research in this southern British area, the Scottish Fishery Board having consented to act in a similar capacity for the northern area. The Hon .Treasurer (J. A. Travers), the Hon. Secretary (E. J. Allen) and G. P. Bidder, together with W. Garstang, were appointed by the Council a committee to inquire as to the best means of providing a suitable vessel for carrying out the investigations and to draw up a scheme of organization for the work. For the investigations in the English Channel it was decided to use the *Oithona*, the acquisition of which vessel by the Association had been made possible by Bidder in 1901. As regards the North Sea investigations the Government were unable to consider the purchase of a ship, and it was necessary to charter. In August 1902 the Secretary reported to the Council that 'Mr Bidder had made an offer to purchase a steam trawler which he would let to the Association upon favourable terms for use in the International Investigations'. The vessel he purchased was a 115 ft. steam trawler, the *Khedive*, which was renamed *Huxley*, in honour of the first President of the Association.

In the minutes of the Association is to be found the following: 'Council wishes to place on record its sense of the value of Mr Bidder's generous assistance in obtaining a vessel for the purposes of the N. Sea exploration.' For this North Sea work the Association rented a house in Lowestoft, and it is to be noted that in each of the years 1903 to 1906 Bidder was appointed to the Committee to visit and report on the work of the Lowestoft laboratory.

In 1905 the Council of the Association were asked to consider whether the work on which they had hitherto been engaged could not be carried out on a reduced scale in future, so as to admit of a substantial sum being allocated to the Board of Agriculture and Fisheries for the collection of statistics. On 28 June 1905 the Treasury sanctioned a grant of £5000 a year for two years' further work on the international investigations, saying 'This sum is less by £500 than the grant hitherto allocated to the Association from votes of Parliament; but my Lords understand that Mr G. P. Bidder has generously offered to return to the Association a sum of £500 a year for two years in respect of the money paid to him for the hire of the Steam Trawler *Huxley*, so that the Association will be enabled through his liberality to carry on its scientific work on the same scale as in the past three years.' The Council considered 'that this offer has been the means of a satisfactory arrangement being made by the Treasury for the continuation of the English scientific work'.



S.S. *Huxley* lying off Billingsgate for the inaugural inspection before starting the International Investigations in the North Sea. (From a drawing reproduced in the *Daily Graphic* of 3 December 1902.)

In 1907 Bidder sold the *Huxley* to the Association on easy yearly terms of payment on condition that, when the Association finally sold the vessel, any profits on the original cost might be kept by him to found a trust for research. In 1909 the *Huxley* was sold with a profit of £750 with which Bidder founded the Ray Lankester Fund to enable selected investigators to work at the Plymouth laboratory.

The *Huxley* did an immense amount of pioneer research, and I have outlined its history at some length because there can be no doubt that this, made possible by Bidder, must have weighed heavily in the Association's favour in its most critical year, 1909. In that year it was proposed that the Board of Agriculture and Fisheries should take over responsibility for English marine research; and eventually the whole of the Association's scientific staff, except for Allen, became government officials. A deputation led by (Sir) Arthur Shipley called on Lloyd George, as Chancellor of the Exchequer, to state the case for continuation of the work under the Association. Although the Government won the day on the point at issue, the Marine Biological Association and its Plymouth laboratory survived, having proved the value of fundamental research as providing necessary background knowledge for fishery investigations.

A vivid account of the early days of the Association and of some financial affairs is given by Bidder himself in his obituary of E. J. Allen in this *Journal* (Vol. 25, p. 671); it is characteristic that there is little mention of his own hand in these affairs.

In the submission of George Parker Bidder's name as the first Honorary Member of the Association at the Annual General Meeting on 4th July 1945 the newly elected President Prof. (Sir) James Gray said: 'We recall with particular gratitude the decisive support which Dr Bidder has given to this Association since he first became a member. During this period the Plymouth laboratory has moved far beyond the objectives which must originally have seemed possible; as each milestone has been reached it has been Dr Bidder's wise foresight, courageous faith and financial aid which made it possible to maintain the advance.' Here followed allusion to the vessels *Oithona* and *Huxley* of which I have already told. Then the President continued: 'Just before the outbreak of the First World War, it became obvious that the buildings at Plymouth were becoming inadequate—particularly for the newer aspects of biology then beginning to attract attention in this country and abroad. In the doldrums after the war Dr Bidder convinced the Council that bold and effective action should be taken, and, once again, made these plans financially practicable. So it has been at each subsequent stage in our history; Dr Bidder's foresight, courage and determination has seen us through. When we survey our present-day activities and gratefully acknowledge the extent to which we owe these to Government support, we must not forget the debt which we owe to Dr Bidder. The early years of this century were a very critical period in our life history and had it not been for his efforts, and his unflinching determination, no amount of Government aid would afterwards have enabled us to avoid disaster. The financial contributions which Dr Bidder has made from time to time amount to a munificent sum, but it has been the time at which they were given and the encouragement which they inspired which have been decisive in the development of the Plymouth Laboratory. Bis dat qui cito dat.'

It was this latter characteristic of right timing which enabled Bidder to play so decisive a part, not only in the affairs of the Marine Biological Association, but in the general development of zoology in this country. Such occasions were, his purchase of the *Quarterly Journal of Microscopical Science*; his formation of the Company of Biologists and resulting saving of the *Journal of Experimental Biology*; and his quick action at the end of the Second World War in persuading the Royal Society to come to the assistance of the Stazione Zoologica at Naples.

Bidder's love for Naples was, perhaps, second only to that for the Marine Biological Association. Reinhardt Dohrn says in a letter to me:

'Among the younger generation of British friends—young as compared to the generation of T. H. Huxley, Francis Balfour, and Ray Lankester—it was G. P. Bidder who had most probably the closest contact and personal friendship with Anton and Marie Dohrn. He must have been frequently at our house during the eighties and the nineties because, young as I was then, I have a distinct recollection of him and his vivid conversation and his characteristic laugh. My parents mentioned him often, him and also the famous, almost classic story, of how he came to take over the bankrupt hotel on the Corso Vittorio Emanuele, which later became such a success as Parker's Hotel.'

Dohrn speaks of Bidder's help to the Stazione between the wars and says:

'It was Bidder's strong wish to keep alive the personal contact between the British biologists and the Zoological Station, and equally alive was his tendency to act as a catalyser of these contacts. Of this, I too have also had proof again and again. But especially so after the last war when a critical situation threatened the Zoological Station and when it seemed doubtful whether the tradition of the Stazione could be continued. Bidder then intervened decisively in more than one case. I am glad to have an opportunity to state this fact here: on 14 October, 1943, scarcely two weeks after the allies had occupied Naples, Bidder—as Chairman of the Marine Biological Association—wrote a letter to the Editor of *The Times* in which he expressed his joy about the news that the Zoological Station had been spared. Many of his fellow countrymen would share his joy because many of them had worked at Naples. And of special value to us here was the fact that he gave vent to his joy "that the Director (friend of us all) was still there in charge in August" (and that this "gave grounds for hope that we may yet see in the Stazione again men of many tongues and more nations working side by side in the fraternity of a common endeavour to understand the living world that surrounds us") which meant that also in the future the Station would go on in the old tradition.

This letter written by Bidder has been of great significance for the fate of the Zoological Station. There was, at that time, a kind of "Reader's Digest", edited in Italian by the Military Authorities, called *il Mese* (the Month). In no. 3, December 1943, Bidder's letter to *The Times* was published in Italian. What that meant for the position of the Zoological Station then, and to myself and my collaborators, need not be emphasized to you. But more was to come: two months later the Allied Military Government informed us that the Royal Society had decided on an emergency grant of £1000.'

Bidder, who was awarded the Sc.D. of Cambridge in 1916, was a prominent figure in biological circles. He was on the Council of the Marine Biological Association from 1899 until his death, a Governor of the Association from 1905, and its President for the years 1939 to 1945. When he resigned from his Presidency the Council instituted the election of Honorary Members of the Association so that they might show their appreciation to Bidder by bringing forward his name as the first to be elected. His last visit to Plymouth was in 1941 during the air raids when the laboratory was badly damaged. The destruction of Plymouth and the haunts he knew so well as a young man caused him great distress.

His colliery interests and knowledge of coal mines made him the obvious secretary for the British Association Committee which reported in 1904 on the probability of *Ankylostoma* (Miner's Worm) becoming a permanent inhabitant of British coal mines in the event of its introduction.

He was President of the Zoological Section of the British Association in 1927. During his year of office he thought that it would be valuable to have intermediate meetings of the section. The Recorder of that day, Prof. Frank Balfour-Browne, being in favour of the suggestion, Bidder and he started to arrange a meeting for January. The Council of the British Association did not, however, approve, so these two started the Association of British Zoologists, of which Bidder was the first Chairman, a position in which he excelled. He was Vice-President of the Linnean Society in 1924 and 1931, and its Zoological Secretary from 1928 to 1931; and an Honorary Vice-President of the Ray Society. He was President of the Devonshire Association in 1929, and of the South Western Union of Naturalists in 1928; to both he gave Presidential addresses proving his wide knowledge and the originality of his outlook. From 1920 to 1927 Bidder gave the course on sponges for Part II students at Cambridge and it was my privilege to be one of the first to listen to these fascinating and stimulating lectures. Bidder loved microscopy, and in his practical teaching he insisted upon the right use of the microscope. It was only by making the best possible use of the instrument that fine details of the cell could be seen, for throughout his work it was the living cell and its complexity that fascinated him. He edited Vosmaer's posthumous *Bibliography of Sponges* which was published in 1928.

As regards his own scientific research, Bidder's two major contributions to knowledge were on the hydraulics of the sponge, and the invention of the bottom-trailer, a bottle so weighted that it was only just heavier than sea water and would drift in bottom currents.

The work on sponges was started at Naples during the years 1887 to 1892 and continued at Plymouth from 1893 to 1896, but the first full report was not made until the British Association Meeting in Hull in 1922, when E. J. Allen was President of Section D. It was published in the *Quart. J. micr. Sci.* the following year. 'For a long time I proposed to myself to make a further series of experiments to clear up doubtful points, but recognizing that I shall not now do so, I have reconsidered all the experiments this year (1922) and recalculated all results and formulae.' He did, in fact, later work for short periods at Plymouth and at Naples, on the currents of sponges, on which he read a paper at the British Association at Leicester in 1933.

It is generally recognized that Bidder was well in advance of his time when he did the research last century which led to his two papers 'The relation of the form of a sponge to its currents' and 'The perfection of sponges'. Their delayed publication until 1923 and 1937 covered the period of the general rise in this country of functional morphology and experimental biology. The papers were models of lucidity; mathematical calculations were relegated to appendices, and the results were available in simple language for all to understand. One of their characteristics was the illustration of details by comparison with graphic everyday phenomena. 'I worked with two calcareous species of sponges, having oscula at the end of tubular prolongations, which reach the size and shape of a child's thumb in the case of *Leucaltis*, and of a child's finger in the case of *Leucandra aspera*'.

While remarking that under the microscope flagella appear to move with the speed of an express train, in fact, he says 'no part of the most rapidly moving flagellum ever attains the rapidity of motion of a snail'. To illustrate that there is no co-ordination in the movement of the flagella in a sponge he says 'A collar cell flagellate surface is comparable mechanically to a seine-net with a number of fishes fixed by their gills in the meshes.'

Again 'The remarkable achievement of the perfected hydraulic organ in sponges is that from this waving of hairs $\frac{1}{100000}$ of an inch in thickness at a mean speed of 7 feet an hour, there is produced an oscular jet with an axial velocity of over half a foot a second (280 times the speed of the flagellum), which in *Leucandra* throws to the distance of 9 inches five gallons a day or a ton in six weeks.' This is a good example of how he stressed the comparison of time and distance in small organisms.

For those species of deep-water Hexactinellida set in a vertical plane with no evolution of pressure chambers: 'Food is brought to them, waste is taken away. For them in their eternal abyss, with its time-like stream, there is no hurry, there is no return. Such an organism becomes a mere living screen between the used half of the universe and the unused half—a moment of active metabolism between the unknown future and the exhausted past.'

The following is very typical: 'A pretty method was arrived at accidentally, when I found the coloured jet marked by dark beads or nodes, caused by my pulse shaking the pipette; the length between any two nodes, divided by three-quarters of a second, gives the core-velocity of that part of the jet.' Bidder once told me how valuable he found a *musca volitans* in his eye for microscopic work: he had calibrated it for different oculars and objectives of his microscope and could use it as a quick approximate eye-piece micrometer for measurements.

His sense of humour was keenly developed, and all his discussions were liable to be punctuated at intervals by that characteristic laughter when he had suddenly noted a humorous aspect. This sense could not be withheld even from his writings. Discussing the ancient history of sponges and climates in his Presidential Address to the British Association Zoology Section at Leeds he said: '...so that 10 or 20 million years would give them as many steps in evolution, to make a flagellate from nothing, as it has taken us to build up a flagellate into that highest of all living creatures, a member of the British Association (section D)'. Or, when writing on flagellar motion 'at higher frequencies the stroke is the shorter, as in a school-master's cane; at lower frequencies the stroke is the longer, as in a fisherman's trout-rod'.

Many of his remarks have deep significance. In an article in the *British Medical Journal* on Senescence he says: 'I suggest that we were not born with infinitely senescent brains and rejuvenated epithelium; rapidity of division in our skin has been evolved to keep it clean and whole; undivided brain cells have been evolved because their function is to remember.' On a visit to his house in 1945 (he was then 82) he took me across his lawn for his daily reading of the maximum and minimum thermometer. On being asked why he kept the thermometer so far from the house he said that it was so that he could daily exercise his memory, as he did not write the readings down until he had returned indoors.

Bidder wrote a number of other papers on sponges besides the two I have specially mentioned. Of these remaining papers Dr M. Burton writes: 'Although fairly numerous, they were all short, but he contrived to include the occasional idea that was of fundamental importance, the most outstanding being his emphasis on the distinctness of the Hexactinellida from the rest of the Porifera, epitomized in his use of the phyla Nuda and Gelatinosa.'

Bidder's invention of the bottom-trailer, in the words of Dr J. N. Carruthers '...represented a powerful means of attack in the study of bottom water movements. Bidder found the important result that the main drift in all his series, when generally summarized, seemed to be in the opposite direction to the migration of the plaice at the same time of year. "So far therefore" he concluded "the experiments confirm the view of those naturalists who suppose that bottom feeders, like trout, tend to move against the current".

Moreover, Bidder expressed the opinion that his evidence favoured the supposition that the percentage of bottles in a given area recovered by the trawl did not differ largely from the percentage of plaice of 21–26 cm in the same area caught by the trawl in the same time. Hence the bottles serve as an instrument for assessing the intensity of trawling because they cannot migrate.

Bidder, in a manuscript given to me, stated that his bottles were trawled up by the fishermen at the rate of 55% per annum.

He deduced the time of onset of the winter drift from south to north in the southern North Sea and of the draught getting into the Skagerrak with the onset of winter. This latter he found makes itself felt by degrees further and further south without drawing on the English Channel water until the end of the year.

Of course, the most significant thing was Bidder's finding that many of his bottom-trailers got cast on the English shore, whereas surface bottles would, for the most part, go across the North Sea. He deduced, regarding the bottom flow, that the isochrones (his word) of the stream-front were shaped on the shoreline; and such a formation of the bottom current at once suggested to him the creeping-in of heavy water. This is of course now well known—that river outflow induces an ingoing bottom set of salt water.'

I have been privileged to see extracts from many of the tributes sent to his family by his friends young and old. While many recalled his culture, his original and fascinating conversation, his child-like curiosity, or his ingenuity, all stressed his courteous and kindly nature. No young student was beneath his consideration, and the numbers, in all stations, whom he helped by word or deed, will never be known. Truly his left hand knew not what his right hand gave.

In conclusion, I cannot improve upon the words of the obituary notice in *The Times* of 1 January 1954: 'But perhaps he brought to the service of humanity and of science qualities rarer and more important than those which have given other men greater reputations. For the timely help and wise advice which he gave to many, both scientists and non-scientists, were guided by unusually understanding sympathy, and were completely unselfish. These qualities had as their natural symbol his unfailing and distinguished courtesy; and that also helped to give its characteristic style to that sturdy bearded figure with white tie, Inverness cape, and quite unconsciously patrician bearing, whose departure many men and women in all branches of study and in all ranks of society will now most sorely regret.'

F. S. RUSSELL

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EXPERIMENTS ON THE HUMANE KILLING OF CRABS

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(Plates I and II)

The main purpose of the work described in this paper was to find a humane way of killing crabs for eating, but the method that resulted from the investigation is applicable also in the biological laboratory, unless it is necessary that the nerve ganglia should be intact.

MATERIAL

The whole of the work was done on the edible crab, *Cancer pagurus* L.

In order to get reliable results, capable of repetition by others, it was necessary to work with fully active crabs, and this could only be done near the sea. The whole of the work reported in this paper was done at the Marine Biological Laboratory, Plymouth, at the end of August 1953.

Crabs brought in by fishermen were placed in a large outdoor aquarium, plentifully supplied with running sea water. No crab was used unless it gave evidence of full normal activity. The first piece of evidence required was that it should make vigorous attempts to avoid capture by a net.

Each crab, when so captured, was brought from the aquarium into the laboratory and subjected to further tests. It was found best to place the crab the right way up in a large basin of sea water. Care was taken to devise tests that would be practical and reliable. A normally vigorous crab gives these responses to stimuli:

Ocular reaction. The crab retracts the 1st and/or 2nd antennae when the hand is passed from one side of the animal to the other above the eyes. (The hand must not touch the animal.)

Antennal reaction. The crab retracts the 1st antennae when the distal (pigmented) end of the middle joint of the protopodite of this appendage is gently touched with a seeker.

Maxilliped reaction. The crab folds the 3rd maxillipeds towards the body and/or brings the chelae towards one another when the 3rd maxilliped is gently pressed away from the body with a seeker.

Pincer reaction. The crab grasps a hard object placed so as to touch the tubercles on the propodite of the chela.

The first two tests provide evidence that the cerebral ganglion is intact, while the third and fourth do the same for the posterior ganglion. These were the tests usually applied, though others were sometimes used. No crab was used in the experiments unless it showed normal responses.

The twenty-five crabs subjected to the main control and experimental procedures are distinguished in this paper by the serial letters A to Y. Details of the experiments with these crabs are given in Table I on p. 18. Two crabs which were treated in such a way that the experiments could not conveniently be summarized in the same table are designated AA and BB.

METHODS

Fishmongers boil crabs in a solution of common salt approximating in concentration to sea water. At the seaside, sea water is sometimes used. In the experiments described in this paper, sufficient tap water was added to 288 g of culinary bar-salt to make 8 l. of solution. This solution (approximately 3·6% sodium chloride solution) was heated in a metal vessel by a large gas burner.

All the crabs used in this investigation, except one, were boiled in this solution, most of them after preliminary treatment intended to render them insensible. It was thought advisable to watch carefully for movements of the appendages during the process of boiling, and the temperature was therefore kept somewhat lower than the actual boiling-point. The mean temperature at the moment of immersion was 96° C. Although the gas-burner was left on, the temperature was usually somewhat lower (mean, 92° C) after the lapse of 6 min, when in every case except one all movement was at an end. The immersion of crabs in this very hot water is called 'boiling' for short throughout this paper.

It was found convenient to hold the animal in tongs, nearly horizontally, the right way up, during immersion. Crab A was placed upside down.

The special procedures intended to render the crabs insensible are described in the appropriate places below.

RESULTS

Controls (no Treatment before Boiling)

Seven crabs, A to G, were boiled alive without previous treatment.

In all cases the time elapsing between the moment of immersion and the occurrence of the last movement was carefully noted.

When an untreated crab is placed in the hot water, the autotomy-reaction occurs immediately in the chelae and walking legs. All these ten appendages are instantly raised and pressed against the edges of the carapace. This movement is complete in about 10 sec. If it is powerful enough, the appendage breaks off at the fracture-plane in the basi-ischium. The greater part of the appendage falls away, either at once or after a short lapse of time. While the appendage is held firmly against the carapace, it cannot move until it breaks. When it breaks, the stump is free to move, and it often does so. There is, however, no continuous movement. On the contrary, all that one observes is an occasional twitch. In general, the reaction to the heat is simply a powerful, continuous contraction of the extensor muscles of the appendages that have fracture-planes; that is to say, the chelae and walking legs.

The following are the periods (in minutes and seconds, to the nearest 5 sec) that elapsed between immersion and the last movement: 0·15, 4·15, 6·50, 2·45, 2·35, 2·45, 0·10 (mean, 2·50).

Although these periods were always measured, they seem to have little value. It seems probable that apart from the contraction of the extensors, movement is not initiated by the nervous system. It seems much more likely that it is caused by the direct effect of the heat on the substance of the muscles. The following experiment was done to test this hypothesis. A crab (AA) was exposed to the vapour of chloroform until it appeared to be dead. On removal from the vapour it made no spontaneous movement, not even a flicker of the 1st antennae or of the flagella of the maxillipeds. No response was given to any of the tests. This crab was boiled in the usual way. No appendage was thrown off by autotomy, but the last movement occurred 2 min 5 sec after immersion. It seems certain that this was merely the result of the shortening of a muscle of a dead crab under the influence of heat.

Movement of an appendage was also occasionally observed to be caused by the expansion and escape of air contained in the branchial chambers.

For these reasons it was concluded that the timing of the last movement was useless, and that it would be best to concentrate attention on the autotomy-reaction, which is governed by the nervous system.

The total number of appendages thrown off by each crab is shown in Table I (p. 18). There is not much difference between one appendage and another in the tendency to be thrown off on boiling.

Among the seven control crabs, only one appendage (a 4th walking leg) was absent by previous injury when boiling started. Of the sixty-nine appendages present, forty-two (61 %) were thrown off on boiling.

Immersion in Strong Salt Solution before Boiling

It was found by Aaser (n.d.) that if lobsters are placed in a strong solution of common salt (350 g to each litre of water), they may subsequently be boiled without showing any other reaction than a single flip of the abdomen. Quite short periods in the strong salt solution sufficed—a minute or even less.

In the present investigation the same method was tried on crabs. Exactly the same salt solution as that used by Aaser was employed. The temperature of the solution was 18–19° C.

Five crabs, BB and H to K, were each placed in strong salt solution for 10 min. The experiments were done separately, so that full attention could be concentrated on the reactions of each animal.

When the crabs were placed in the strong salt solution, the first antennae were at once strongly retracted and could therefore give no response. In general, the animals then at first remained still. After a short lapse of time (half a minute or so) some movement of the walking legs started, with feeble locomotion in some cases. No response was given to stimulation of the maxillipeds, and the pincer reaction was very weak. Spontaneous movement had ceased before the 10 min had elapsed.

TABLE I. DEGREE OF AUTOTOMY AFTER DIFFERENT TREATMENTS

				No. of autotomy appendages	
Crab	Sex	Width of carapace (cm.)	Treatment before boiling	Absent before boiling	Lost by autotomy on boiling
A	♀	12	None (control)	0	2
B	♀	16	None (control)	0	8
C	♀	18	None (control)	0	9
D	♂	14	None (control)	0	7
E	♂	14	None (control)	0	7
F	♀	16	None (control)	0	3
G	♀	15	None (control)	1	6
H	♀	16	Immersion in strong salt solution	1	4
I	♀	16	Immersion in strong salt solution	0	10
J	♀	18	Immersion in strong salt solution	0	10
K	♂	16	Immersion in strong salt solution	0	9
L	♀	19	Immersion in tap water	0	6
M	♂	11	Gradual increase of temperature to boiling	0	3
N	♀	16	Piercing of brain at 85°	0	5
O	♀	17	Piercing of brain at 85° and v.n.-m. at 110°	1	1
P	♂	12	Piercing of brain at 85° and v.n.-m. at 110°	0	0
Q	♂	14	Piercing of brain at 85° and v.n.-m. at 110°	0	0
R	♂	14	Piercing of brain at 85° and v.n.-m. at 110°	1	0
S	♂	12	Piercing of brain at 85° and v.n.-m. at 110°	0	2
T	♀	15	Piercing of brain at 85° and v.n.-m. at 110°	0	1
U	♂	14	Piercing of v.n.-m. at 85°	0	0
V	♀	16	Piercing of v.n.-m. at 85°	1	0
W	♂	16	Piercing of brain at 60° and v.n.-m. at 85°	0	0
X	♀	15	Piercing of brain at 60° and v.n.-m. at 85°	0	0
Y	♂	17	Piercing of brain at 60° and v.n.-m. at 85°	0	0

The piercing angles given are only approximate. v.n.-m.: ventral nerve-mass.

The crab BB was returned to sea water. It soon gave the ocular and antennal reactions again, but irreparable damage had been done, and it was found to be dead about 6½ h later.

The other four crabs (H to K) were boiled immediately after they had been taken from the strong salt solution. Before the experiment they had thirty-nine appendages capable of autotomy, only one walking limb of one animal having been absent; thirty-three appendages were thrown off during boiling. The percentage of autotomy, 85 %, was even higher than in the controls.

It is clear that short immersion in strong salt solution is not a suitable method for rendering crabs insensible. The difference from lobsters is no doubt caused by the far greater protection of the gills in the crab. The effect of the strong salt is only slowly felt if the respiratory current is weak or intermittent, or if there is much air in the branchial chambers.

The American crab, *C. magister*, which lives below low-tide mark in Pacific seas just as *C. pagurus* does in ours, and is thus not subjected in nature to any but very small changes in the osmotic pressure of the surrounding medium, is unable to regulate the osmotic pressure of its blood against water of higher salinity than the sea (Jones, 1941). If *C. pagurus* were left for a sufficient period in strong salt solution, it would eventually die from increase of the osmotic pressure of its blood, as indeed crab BB died; but there is no likelihood that this slow death would be painless.

Immersion in Cool Tap Water before Boiling

It was thought worth while to find whether a crab could be rendered insensible quickly by immersion in tap water.

The water was at 17° C. The crab (L) was much more active than the ones placed in strong salt solution. The respiratory current was strong. Some unco-ordinated movement occurred (the animal grasped a maxilliped with the chela of the opposite side, and later it moved its maxillipeds from side to side). The animal moved about from time to time.

At the end of 10 min the crab was boiled. Of the ten autotomy-appendages, six were thrown off.

From a study of *C. pagurus* placed in water of lower salinity than that of the sea, Hukuda (1932) concluded that the gills are permeable to salts in these circumstances, so that the saline concentration of the blood drops. The placing of the animal in tap water must result in eventual death from loss of salts from the blood, but the experiment recorded in this paper shows that the process is not a quick one.

Gradual Increase of Temperature to Boiling

According to Sinel (n.d.), if a lobster is placed in weak salt solution and the temperature is gradually raised, the animal becomes lethargic and dies at about 27° C. The heating to this temperature took about 3 min in Sinel's experiments. Sinel recommends this as a humane way of killing both lobsters and crabs, though he appears not to have made experiments with crabs. This result was supported by an experiment carried out on lobsters under the auspices of the Trondhjem Society for the Protection of Animals (Anon., n.d.).

Aaser (n.d.) put lobsters in a weak solution of common salt at 9° C and gradually heated the water. They moved about at first, but their activity gradually decreased and they died between 38° and 45° C. Aaser found that in general a lobster immersed in water at 26° C continues to respond to stimuli for about $\frac{3}{4}$ h.

In the present investigation, a crab (M) was placed in a 3·6% solution of common salt at 19° C. The solution was gradually warmed. The temperature of 27° C was reached in about 7 min. The crab was still active. The temperature was increased gradually till boiling point was reached in 28 min. The crab was active at 26° C and continued to move about as the temperature rose higher. It attempted to escape by pushing backwards against the sides of the vessel. At 32° C the movements began to become unco-ordinated. At 34° C it was much less active and there was no locomotion; both chelae were thrown off. The 1st walking limb of the left side was subsequently lost. At 40° C, reached in 8½ min, the last movement was seen (very slight change of position of the last walking limb); no response was given to stimuli.

Crabs only perform autotomy when they are vigorous, and it is probable that if the experiment had been performed on a specimen obtained from a fishmonger, no limb would have been thrown off. However, this method of killing crabs does not commend itself on humanitarian grounds.

Piercing the Nerve Ganglia before Boiling

Some years ago I visited Billingsgate fishmarket and witnessed the 'sticking' of two crabs before boiling (Baker, 1949). A steel awl mounted in a wooden handle was used. The animal was laid on its back and the point of the awl was pushed through the exoskeleton in two places, referred to by the operator as the 'mouth' and 'back passage'. I noted where these points were. The anterior one was the very small depression in the middle line at the posterior end of the sternum of 1st antennal segment, immediately in front of the epistoma (Pl. 1, fig. 1). The posterior one was the large depression in the middle line at the posterior end of the sternum of the segment of the chelae (Pl. 1, fig. 2). It was evident that the awl would necessarily damage the two main nerve centres of the body, the brain (fused cerebral ganglia) and ventral nerve mass. What I saw encouraged me to believe that it would be useful to investigate this method.

I made a special dissection to find out in what direction the awl should be pointed in order to reach the two ganglia from the depressions mentioned.

The animal must be placed as horizontally as possible on its back. It would be best to have a bench with shaped hollows in it, suitable for holding crabs of various sizes horizontally. However, it suffices in the laboratory to twist a duster into a roll and to shape this in a ring, against which the edges of the carapace can rest.

The awl must of course be kept in the sagittal plane of the animal. The angle at which it is held will be described as follows. If it is held vertically, the angle is 90°. If the handle of the awl is moved forwards, a lower angle is recorded, till 0° would be reached if the point of the awl were directed horizontally backwards.

I have had a special awl made for sticking crabs. The steel point is fine, so as to make as small a hole as possible and thus allow little blood to escape. It is 25 mm long. This length is designed to reach easily the ventral nerve-mass of the largest crab. The wooden handle is 30 cm long. This length is designed to make it possible to pierce the largest crab without the animal being able to reach one's fingers with its pincers. With a crab of average size, a push of about 4½ mm would suffice to penetrate the brain, while about 9 mm would be required for the ventral nerve-mass. A push of 13 mm should suffice for the ventral nerve-mass of the largest crab. The awl cannot be inserted up to the hilt, because the latter will not enter the depression in the sternum of the segment of the chela; that is why the steel point is made longer than 13 mm.

It is not necessary (nor indeed possible) to observe the piercing angles very exactly, for the brain is close to the point of the awl when one starts to push, and the ventral nerve-mass, though deeper, is much larger.

When the piercing has been done, the handle of the awl should be moved about slightly in all directions, to complete the damage to the ganglia.

In the first experiment (crab N), the brain only was pierced, at about 85°. The antennal response was now no longer given, but the animal naturally still gave the maxilliped and pincer responses. The crab was at once boiled. It lost five of its ten autotomy limbs. Destruction of the brain cannot prevent autotomy, for this process is mediated by the ventral nerve-mass, as Frederic showed long ago (1882).

Both ganglia of six crabs (O to T) were pierced in the next series of experiments. The brain was pierced at about 85°, the ventral nerve-mass at about 110°. No maxilliped or pincer responses were observed in any of these crabs after piercing, but there was some slight spontaneous flickering of the flagella of the maxillipeds of some of them. There was also at first some spontaneous twitching of the distal parts of the 1st antennae. One of the crabs (P) gave a very slight antennal response, another (S) an ocular response.

Each of these six crabs was boiled directly its responses had been observed. Two of them each lacked one chela before boiling; there were thus fifty-eight autotomy limbs among the six crabs. Of these, four (7%) were thrown off during boiling. Three of the six crabs lost no appendage.

These results were encouraging, but both the responses and the autotomy showed that the direction of the awl was not quite accurate. Another dissection was made, and it was decided to try about 60° for the brain and about 85° for the ventral nerve-mass. In piercing the latter at 85°, it is important not to try to insert the point of the awl into the very bottom of the hole in the sternum, for the depression turns posteriorly, and the awl will have to be held at a lower angle than 85° if its point is to touch the bottom. One should hold the awl at about 85° and place it as far back in the hole as is consistent with the maintenance of this angle.

In two crabs (U and V), the posterior ganglion only was pierced. No maxilliped or pincer reaction was given, but V gave the ocular reaction and at first showed some spontaneous twitching of the distal parts of the 1st antennae.

This was only to be expected, since the brain was intact. On boiling, neither crab lost any appendage.

In a final series of experiments, both the brain and the ventral nerve-mass of three crabs (W to Y) were pierced in the new directions (Pl. II, figs. 1 and 2). These new directions appeared to be correct, for none of these three crabs gave any response to stimuli. There was, however, some slight spontaneous movement of small parts, such as has already been mentioned: namely, a twitching of the distal extremity of the 1st antennae, and a flickering to and fro of the exopodites of the maxillipeds. In addition, crab Y slightly moved its mandibles and the basal parts of its maxillipeds. It will be recalled that the mandibles receive some innervation from the little paraoesophageal ganglia, which cannot be pierced from outside the animal.

Each of the crabs W to Y was boiled directly its responses and spontaneous movements had been noted. No appendage was absent before boiling. Of the thirty available appendages, none was lost on boiling.

The five crabs U to Y possessed forty-nine autotomy limbs before boiling. The ventral nerve-mass of each of these crabs was pierced at about 85°. It is to be noted that not a single limb was lost on boiling.

From a culinary point of view, it is desirable to prevent the escape of blood when the crab is pierced, partly because the blood coagulates on the surface of the animal when it is boiled and thus disfigures it, and partly because it is thought that the taste of the crab would be affected if blood were lost.

Care must be taken when piercing the brain of a small crab, or the point of the awl will pass through the dorsal part of the carapace and thus allow the escape of blood.

If the piercing is done carefully, with a fine-pointed awl, little blood should be lost. It is a good plan to fold the abdomen back into its normal position after piercing the ventral nerve-mass, and to fix it there by tying a string round the animal. The posterior awl-hole will thus be closed, and since air cannot enter it, blood cannot easily escape from the anterior hole.

Note on Accidental Autotomy

Two of the crabs (O and R) threw off a chela each at or immediately after the piercing of the ventral nerve-mass at 110° (before boiling). Autotomy did not occur with any of the five crabs in which this ganglion was pierced at 85°. Frederic (1882) remarks that on one occasion autotomy occurred when he applied an electric shock to the ventral nerve-mass of *Carcinus*.

DISCUSSION

The evidence from responses to stimuli, and especially from autotomy, suggests that the best method of rendering a crab insensible is to pierce the brain and ventral nerve-mass with an awl in the way directed.

The experiments of Bethe (1898) showed that the brain of *Carcinus maenas* is a reflex-inhibiting organ, which to a large extent controls the adaptive responses of the animal. Those who associate cephalic dominance with cephalic consciousness will be inclined to pierce the brain before the ventral nerve-mass. If so, the appendages that are innervated by the ventral nerve-mass will move actively until the latter has been pierced.

It is fortunate that the method that seems best from the point of view of animal welfare should also commend itself to fishmongers and canners, because it enables crabs to be boiled without losing appendages, however vigorous they may be, and without the waste of time involved in other methods that have been suggested.

The technique of piercing could be taught in 15 min to any person of ordinary intelligence who was accustomed to handling crabs. Preliminary demonstrations could conveniently be given on a preserved specimen.

It is particularly to be noted that a method that is suitable for the crab may be quite unsuitable for the lobster, and conversely. This is because the crab has only a single ventral nerve-mass instead of a chain of twelve separate ganglia, while the gills of the lobster are much more directly exposed to the environment than those of the crab.

The work was carried out under the auspices of The Universities' Federation for Animal Welfare, and with the Federation's financial support. I thank the Director and Staff of the Marine Biological Laboratory, Plymouth, for helping me in every possible way, especially by ensuring a regular supply of very vigorous crabs. Mr P. L. Small took the photographs (Plates I and II).

SUMMARY

The following methods intended to render crabs (*Cancer pagurus*) insensible before boiling were tried: immersion in strong salt solution, immersion in tap water, gradual increase of the temperature to boiling, and piercing of the brain and ventral nerve-mass with a steel-pointed awl. The responses of the crabs to stimuli, and especially the autotomy reaction on boiling, were carefully observed. The experiments showed that the best method is to pierce the brain and ventral nerve-mass. It is important to hold the awl at the proper angles when inserting it in the two positions.

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EXPLANATION OF PLATES

PLATE I

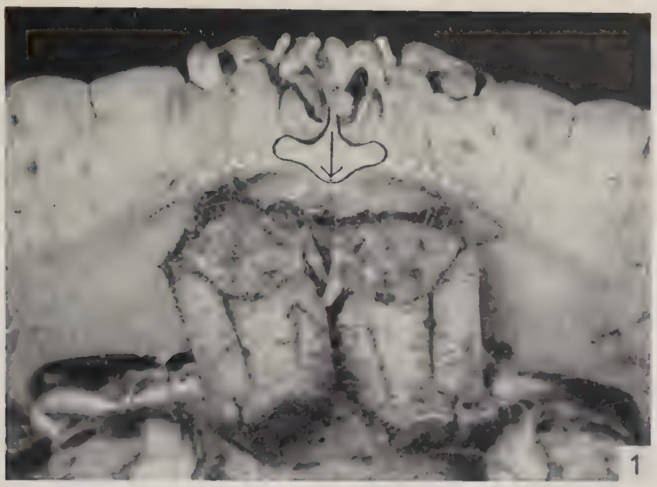
Fig. 1. The anterior end of a crab, in ventral view. The sternum of the segment of the 1st antennae has been outlined in ink in the photograph. The arrow points to the small depression in which the point of the awl must be inserted to pierce the brain.

Fig. 2. Ventral view of a crab. At the posterior end of the sternum of the segment of the chelae is the large depression (marked by an arrow) in which the point of the awl must be inserted to pierce the ventral nerve-mass.

PLATE II

Fig. 1. Piercing the brain of a crab with the specially designed awl.

Fig. 2. Piercing the ventral nerve-mass.



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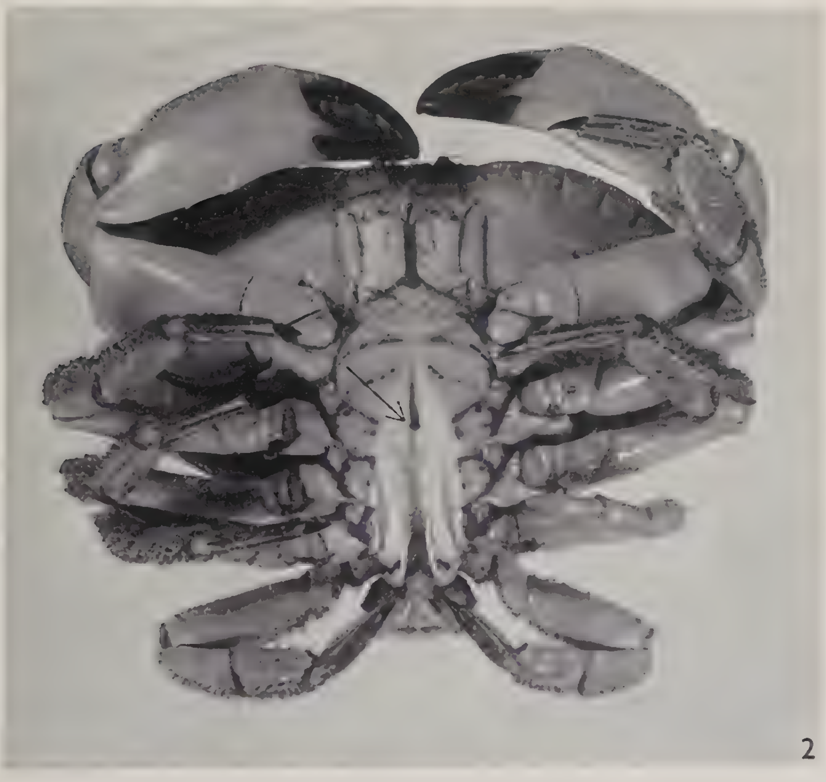

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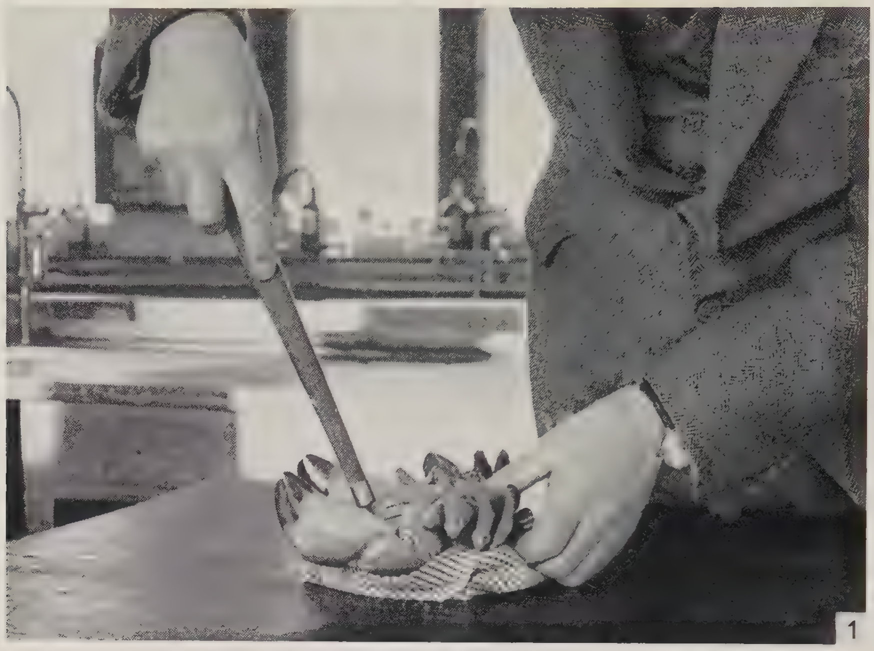

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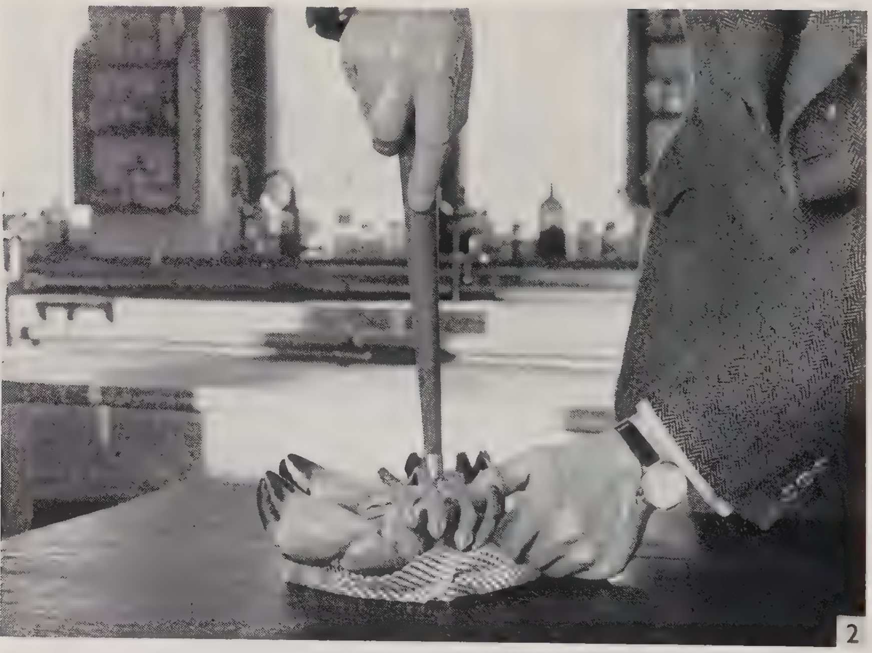

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ODOSTOMIA AS A PEST OF OYSTERS AND MUSSELS

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(Plates I and II and Text-fig. 1)

A recent study by Fretter & Graham (1949) has shown that members of the family Pyramidellidae are ectoparasites, each species feeding on a particular host, usually a tubicolous polychaete or a lamellibranch, by piercing the body wall with the buccal stylet and sucking blood, and perhaps tissue debris, by means of the buccal pump. A list of hosts, each with its specific parasite, was given.

Subsequently, Cole (1951) recorded the presence in oysters of a species, *Odostomia eulimoides* Hanley, which had been observed by Fretter & Graham, and recorded previously by Jeffreys (1867), as feeding on *Pecten maximus* (Linné) and *Chlamys opercularis* (Linné). The *Odostomia* were lodged in small pockets inside the ventral margin of the shell of 2-year-old native brood oysters dredged from near Paglesham in the River Roach, Essex. The pockets were formed as a consequence of the withdrawal of the mantle in response to the irritation caused by the proboscis of the parasite during feeding.

It was suggested that, although *Odostomia* could not be regarded as a major parasite of oysters, its attack must result in considerable irritation, some loss of condition, and possibly small permanent malformations of the shell.

In December 1953, Mr J. E. Francis, foreman of the Colne Fishery Board, brought to the Burnham Laboratory some oysters of marketable size, i.e. 5–6 years of age, taken from the Pyefleet Channel, River Colne, which appeared to be suffering from the cumulative effects of attacks by *Odostomia*. Many of the oysters were malformed and some were dying. Oysters in a similar condition had been noted about 7 years previously. Further researches into the incidence of the parasite and its effects on oysters form the basis of this paper.

We are much indebted to the Colne Fishery Board, and in particular to Mr Francis, for material and information. We are also indebted to Dr Vera Fretter for the identification of the species of *Odostomia* found during the investigations.

OBSERVATIONS

Odostomia *in Oysters*

A sample of forty oysters was received from the Pyefleet Channel in January 1954. They had been noticed and removed by oystermen during normal dredging operations from among several thousand oysters of marketable size. They were 5–6 years of age, and 76 mm in average diameter. These oysters were specially selected as having some malformation of the edge of the shell, and it was estimated by Mr Francis that they occurred once or twice in every 2000 oysters. Of the forty oysters received, all showed symptoms similar to, but far more serious than, those recorded by Cole (1951) as resulting from an attack by *O. eulimoides*. The presence of a small gastropod was noted, and this was found to be the same species of *Odostomia*. In one dying oyster no less than seven *Odostomia* were found. In others one, two or three were present. In some no parasites were found, but, since the oysters had been collected several days before and kept alive in baskets in the oyster pits, and had been handled frequently during dredging and sorting, it is not surprising that they had been lost.

In less severe cases the *Odostomia* were observed in small characteristic pockets usually on the ventral or anterior margin of the oyster (Pl. I, fig. 1). These were similar to those described by Cole (1951). Prolonged irritation by a larger number of *Odostomia*, however, causes a much more violent response by the oyster. The pockets become merged to affect almost the whole edge of the shell, and penetrate farther towards the adductor muscle. The edges of the pocket are marked by numerous thin, approximately parallel, laminae of shell substance, marking the successive positions of the withdrawn mantle edge as the oyster had attempted to evade the probing of the parasite. The margin of the shell becomes thickened, in the worst cases being approximately 1 cm wide with double or triple lips (Pl. 1, fig. 2).

In the most severe cases, the constant irritation appears to interfere with the normal metabolism of the oyster. Great ridges of shell substance are laid down following the path of the withdrawn mantle. Finally the point of attachment of the adductor muscle is reached, but the process continues, with the result that the adductor muscle is almost completely covered by a thorn-like ridge of brownish deposited shell material (Pl. I, fig. 3). Attack from the ventral margin, as normally occurs, will first affect the catch component of the adductor muscle and will cause the oyster to gape. In this condition, sand and silt will be driven into the mantle cavity with resultant suffocation and death. Such a condition was, in fact, commonly observed among the severely affected oysters. In one severe instance, only a narrow band of one-tenth of the adductor muscle remained fully functional. This displacement of the adductor muscle may occur inside either valve of the oyster (Pl. I, fig. 3; Pl. II, fig. 4), but is most usually found in the flat valve. Sometimes, the two valves are affected simultaneously, resulting in a condition in which it is virtually impossible for the oyster to close. In most of the severe cases it was possible to follow the development of the attack by the presence of successive layers and ridges of shell which led back to the damaged adductor muscle. Occasionally, however, although the margin of the oyster shell showed severe pockets, a thorn-like growth was present intruding into the adductor muscle with no connecting layers of similar material between it and the pockets (Pl. II, fig. 5). The impression received was one of severe irritation of the oyster, with consequent strain on the adductor muscle, which had manifested itself in a violent disturbance of its metabolism.

In several of the oysters examined, retraction of the mantle, accompanied by thickening of the edges of the shell, had taken place to such an extent that there had been room for settlement of mussel spat inside the margins of the two valves. The mussels had grown, causing the valves to part even more, finally producing the effect shown in Pl. II, fig. 6.

Of the forty oysters examined in this sample, twenty were so severely affected that the adductor muscle was damaged, and of these, eleven were dying or already dead from the effects of *Odostomia*. It should be mentioned that none of these oysters showed evidence of shell disease, which occurs very rarely in the River Colne, but which when present produces in its final stages effects of a somewhat similar nature in oysters. Occasionally severe attack by *Polydora* may produce blisters on the shell which resemble the pockets caused by light attack by *Odostomia*. With practice, a substantial proportion of oysters affected by *Odostomia* may be detected in the field by the thickening and distortion of the shell edge. However, the superficial resemblance between the condition it causes, in the early stages of attack, and damage by other enemies, such as *Polydora*, has delayed the recognition of the parasite on beds where it is now known to occur.

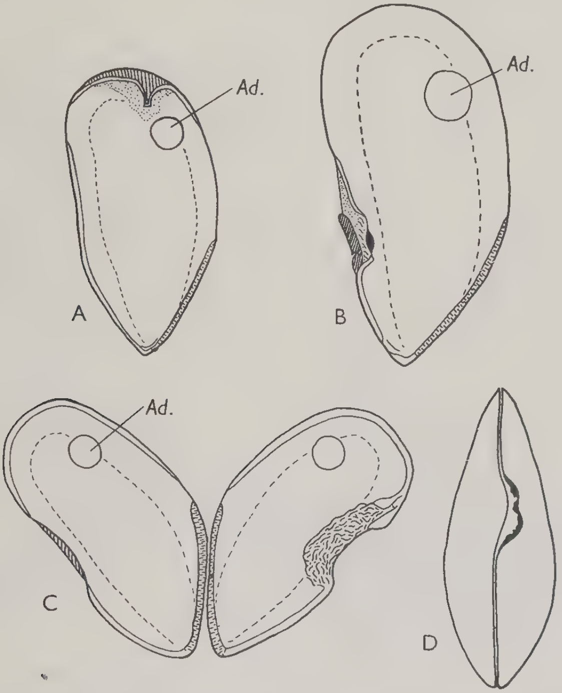
The appearance of such badly affected oysters on the Colne Fishery amongst those intended for market caused some concern. It was decided to investigate the frequency of the occurrence of *Odostomia* in younger oysters on the grounds in the River Colne from which oysters intended for market are taken for final fattening in the Pyefleet Channel. A random sample of 3-year-old brood oysters was dredged, isolated carefully, and examined for the presence of *Odostomia*. In ten, of fifty-four oysters, *Odostomia* was actually present, each of the oysters showing retraction of the mantle and typical pocketing of the shell margin. In six others there was definite evidence, and in four slight evidence, that *Odostomia* had been present. A maximum of three *Odostomia* was found in one oyster, and a total of fifteen in the ten oysters. Three additional *Odostomia* were found in the container. This represents a minimum of 30% incidence of *O. eulimoides* in 3-year-old brood oysters at the lower end of the oyster grounds in the River Colne. Samples from higher upstream indicate that *Odostomia* occurs less abundantly there, but the full pattern of its distribution has not yet been determined.

The evidence that *Odostomia* attack can cause the death of some oysters, and may render others unfit for consumption, led to a search for the parasite when examining oysters from other areas. A feature of its occurrence is its appearance in unexpected circumstances, which has led to the belief that the general incidence of *Odostomia* in oysters must be high. For example, the first oyster opened in the River Colne for a condition measurement contained an *Odostomia.* A small brood oyster from Creeksea, River Crouch, examined for drilling by *Urosalpinx*, contained *Odostomia.* The first oysters opened at Fambridge, River Crouch, and in Salcombe harbour, for other reasons, each contained *Odostomia.* In the River Crouch, 17% of the brood oysters from Brickfield Bight, Bridgemarsh Island, a ground approximately mid-way between the head-waters and the mouth, were infected, while 8% of the brood oysters taken from the Shop Laying, Paglesham, River Roach, also contained *Odostomia.*

O. eulimoides has now been recorded in oysters from Creeksea, Brickfield Bight and Fambridge in the River Crouch, and Shop Laying and Common Shore in the River Roach. An oyster from Salcombe harbour contained a pyramidellid which proved to be a different species, *Chrysallida obtusa* (Brown) which is here recorded for the first time in oysters.

Odostomia *in Mussels*

Information had been received from Dr E. W. Knight-Jones that *Odostomia scalaris* (Macgillivray) was present in large numbers on *Mytilus edulis* (Linné) from the pontoon of Menai Bridge pier. It was thought that an investigation of these mussels might provide a useful comparison with the effects of *Odostomia* on oysters. A sample of 200 mussels, kindly despatched by rail by Dr Knight-Jones, was examined for the presence of *Odostomia* and for any retraction of the mantle comparable with the condition found in oysters. Retraction of the mantle to form a pocket had occurred in eight of the mussels, and, although the *Odostomia* were not present inside the shell, there is little doubt that they had been the cause. The pockets were strictly similar in form to those described in oysters (see Text-fig. 1). Each occurred usually on one valve, while the other valve had overgrown slightly in an effort to complete the gap. The pocket was then just visible externally as a slight twist in the shell margin. Forty-two *O. scalaris* were found on the outer surfaces of the mussels and in the container. Several of these were kept alive in an aquarium tank with small *Mytilus*, and after a short time were found to have taken up a feeding position. Each *Odostomia* was attached to the outer edge of one of the valves with its proboscis protruding into the siphonal aperture of the mussel, and they remained in this position for several days. The length of the proboscis was such that it could have produced the retraction of the mantle without actual entry of the *Odostomia* between the valves as occurs in oysters. This, combined with the fact that the mussels had undergone a long rail journey, may explain why no *Odostomia* were found inside the shells of the mussels.



Text-fig. 1. A, B, Inner surfaces of the left valves of two mussels showing 'pockets' caused by *Odostomia*; A, in posterior margin, B, in ventral margin. C, inner surface of mussel attacked by *Odostomia* at ventral margin. D, ventral margin of mussel C. *Ad.*, adductor muscle.

Discussion

In the present investigation up to seven *O. eulimoides* were found in one oyster, but, since many parasites are lost during dredging and handling, it is likely that still more may occur in large oysters. Cole (1951) found two pairs occupying separate pockets in small 2-year-old oysters from the River Roach, Essex, but the damage caused was not severe. It may be conjectured, therefore, that the very serious damage here described is either produced by substantially more than the maximum number so far recorded, or by attack by smaller numbers over a period of years.

It is probable that in the first stages of attack the *Odostomia* attach themselves to the lips of the shell and probe the edges of the mantle with their long probosces. Later, as a pocket is formed by the deposition of new shell along the line of the retracted mantle edge, near the site of attack, the *Odostomia* are able to move inside the shell and live more or less permanently within the oyster. It is clear, however, that they must leave the pockets at times, e.g. when copulation takes place, and in an aquarium tank *Odostomia* have been noticed moving over the outer surface of oysters which had obviously suffered from attack.

The presence of *Odostomia* in the edges of the shells of oysters during the early stages of attack and their periodical excursions from the pockets to the outer surface of the shell, and no doubt to other oysters, explains the relative infrequency of parasites in samples of oysters which have been dredged and handled before examination.

Cole (1951) recorded developing spawn in the pockets in June, but none has since been found in this situation. Possibly it may be deposited also in crevices on the shells of oysters.

The list of hosts from which members of the Pyramidellidae had been recorded (Fretter & Graham, 1949) included:

Chrysallida spiralis	from	*Sabellaria* spp.
Odostomia unidentata	,,	*Pomatoceros triqueter*
O. lukisii	,,	*Pomatoceros triqueter*
O. scalaris	,,	*Mytilus edulis* (small only)
O. eulimoides	,,	*Pecten maximus* and *Chlamys opercularis*
O. trifida	,,	*Mya arenaria*
Turbonilla jeffreysii	,,	some coelenterate, probably *Halecium* sp.

To these may now be added *Odostomia eulimoides* and *Chrysallida obtusa* from *Ostrea edulis* L. (The nomenclature used follows Winckworth, 1932.)

It appears that the Pyramidellidae may be less specific in their choice of host than has been suggested.

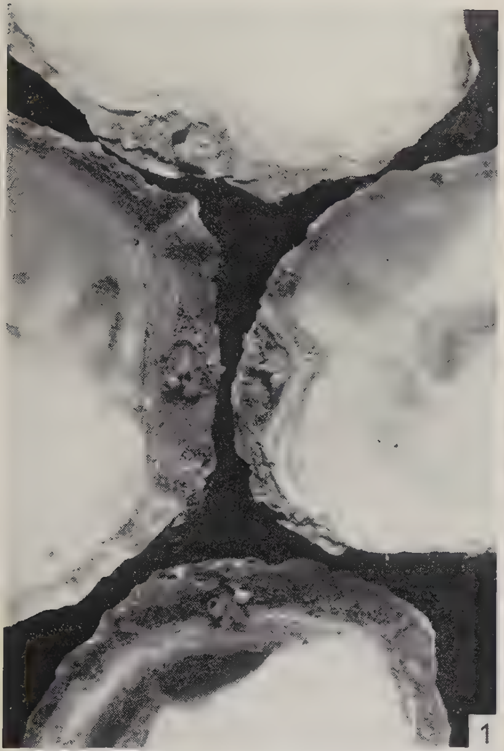
Odostomia, as a parasite of oysters, has some practical importance, not only because of its frequency in young oysters whose growth is likely to be retarded. It has been shown that full-grown oysters may be seriously weakened and killed and it would be inadvisable to include obviously infected oysters in a market consignment, since the discovery of a number in the condition illustrated might prejudice future sales. No measure of control can so far be suggested other than the destruction of large oysters which show the multiple-lipped condition and are light in weight, suggesting low flesh content.

Summary

A description is given of serious damage to oysters of marketable size taken from the River Colne, Essex, caused by *Odostomia eulimoides*. An incidence of 30% infection was found in 3-year-old oysters.

Chrysallida obtusa is recorded for the first time as a parasite of oysters.

The effects of *Odostomia scalaris* on *Mytilus edulis* are similar to those described in oysters resulting from attack by *Odostomia eulimoides*.



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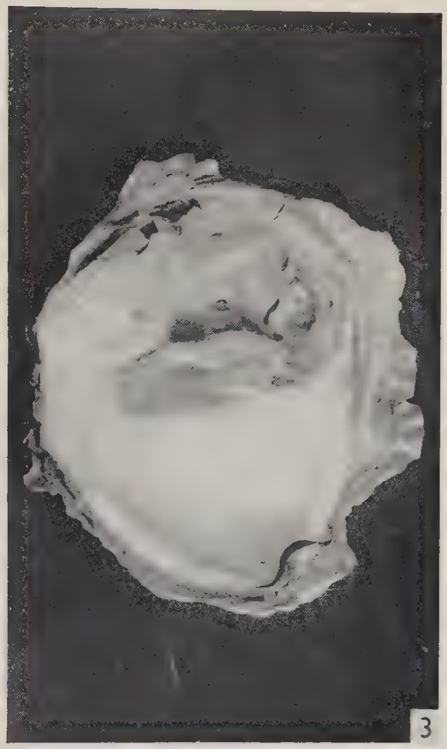

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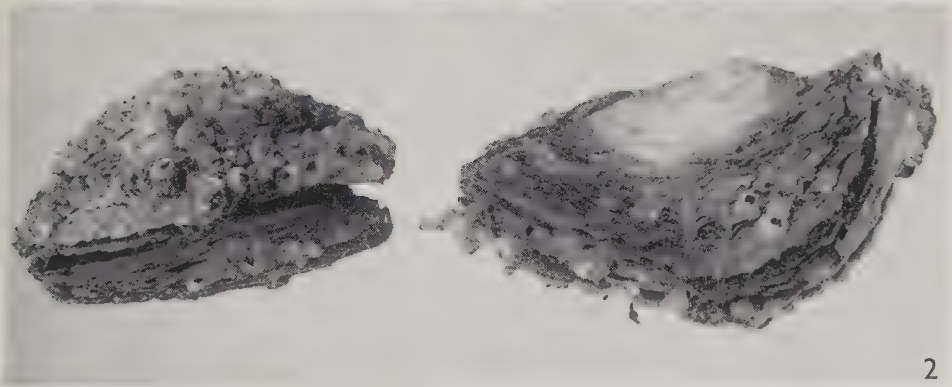

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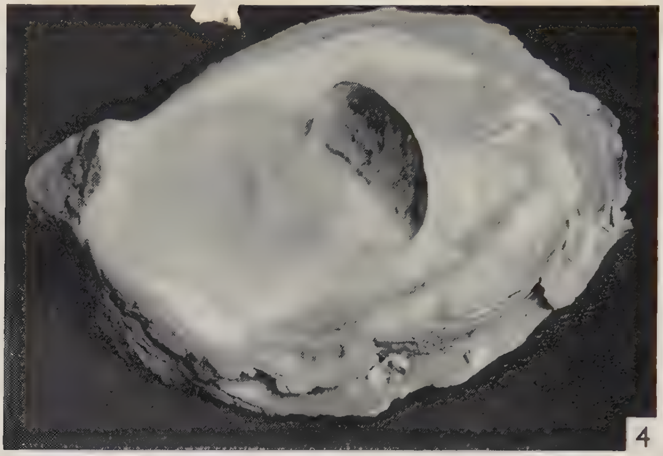

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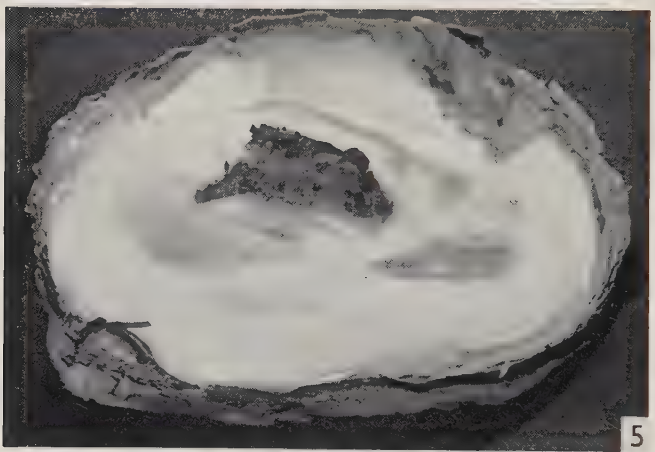

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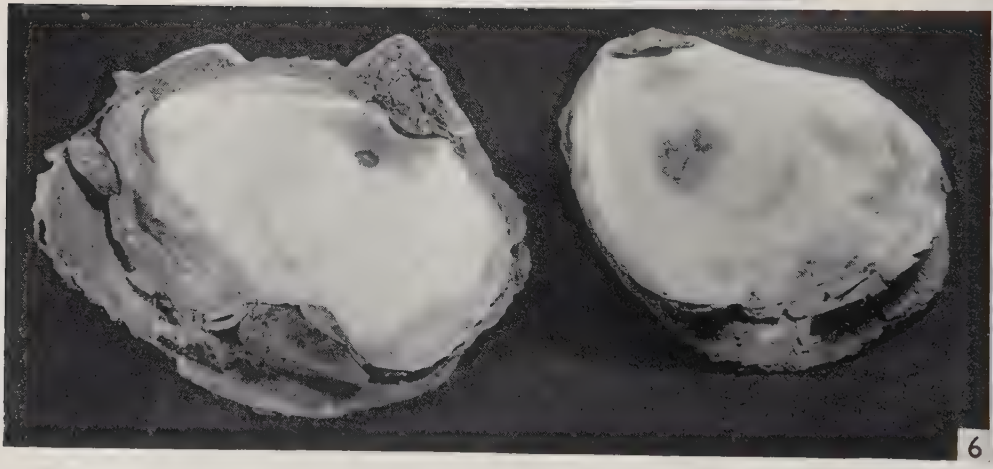

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EXPLANATION OF PLATES

Plate I

Fig. 1. Shells of *Ostrea edulis* showing *Odostomia in situ* in characteristic marginal 'pockets'.

Fig. 2. Ventral margins of two oysters thickened as a result of *Odostomia* attack.

Fig. 3. Inner surface of the left valve of an oyster with adductor muscle attachment affected by severe attack by *Odostomia*.

Plate II

Fig. 4. Right valve of an oyster attacked by *Odostomia*.

Fig. 5. Right valve of an oyster showing typical *Odostomia* 'pocket' and affected adductor muscle attachment.

Fig. 6. Oyster shells showing effects of severe *Odostomia* attack and settlement of mussels between the valves.

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SALINITY VARIATION IN INTERSTITIAL WATER OF SAND AT KAMES BAY, MILLPORT, WITH REFERENCE TO THE DISTRIBUTION OF *NEREIS DIVERSICOLOR*

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(Text-figs. 1–5)

The sheltered beach of Kames Bay, Millport, Isle of Cumbrae, Scotland has been the scene of several important studies on intertidal zonation (Stephen, 1929; Elmhirst, 1931; Watkin, 1942). The last paper is most comprehensive and records in detail the zonation of over twenty species of animals. In the course of the present study I have found Watkin's account very accurate, but incomplete in respect to salinity variation in the interstitial water of the sands. My attention was initially directed to Kames Bay in a search for a population of *Nereis diversicolor* O. F. Müller inhabitating an essentially 'marine' environment in respect to salinity. The suitability of Kames Bay was suggested by a statement of Watkin (1942, p. 558):

> The salinity of the tidal water may be expected to agree with that of the waters of the Firth and to show little or no variation from high to low water, and to be about 32 parts per thousand....The extent of the fresh-water influence in Kames Bay is very slight. A small stream crosses the beach on its western edge, but the line of sampling is some distance from it.

And on p. 544:

> the volume of water of this stream is small and the effect over the whole bay cannot be considered as seriously affecting the type of fauna.

The present report will give evidence that other sources of fresh water in Kames Bay, apart from the small stream mentioned above, are not insignificant, and that the distribution of *N. diversicolor* on the beach may be correlated with the occurrence of brackish water.

As shown in Fig. 1, the western third of the beach is subject to the run-off of a smaller and a larger freshwater stream issuing from culverts in the sea wall. In October 1953, samples of interstitial water were taken along a transect (A) running seaward across the slope affected by these freshwater rivulets. *N. diversicolor* was found to occur in rather uniform numbers along this line, from a point 35 m from the sea wall (i.e. below the high-water neap-tide level) out to 90–100 m. The chloride of the interstitial water varied from

5 parts per mille at 35 m to 16‰ at 100 m (that is, salinities of 9 and 29‰ respectively). These values were maintained beneath nearly fresh overflowing stream water during low tide (at 100 m the flowing surface stream had a chloride of 2·4‰). Such a situation has been repeatedly observed in estuarine environments (Reid, 1930, 1932; Alexander, Southgate & Bassindale, 1932). The broad distribution of *N. diversicolor* contrasted, however, with the narrow zonal grouping reported by Watkin, and seemed possibly related to the wide zone of freshwater influence. As a check of this supposition a second transect (B) was established near the centre of that two-thirds of the beach presumed to be free of freshwater influence. The results of preliminary sampling on transect B showed the expected sharper zonal grouping of *N. diversicolor*, but also an unexpected band of low interstitial salinity extending along the beach in the zone of greatest abundance of the worm. This finding prompted a more careful examination of this part of the beach and of the salinity profile of the interstitial water.

General Description of the Beach

In October 1953, the beach presented a distinct 'upper slope' of loose sand with windrows of debris and cast wrack, commencing close to the sea wall, approximately at the high-water mark of the highest spring tides (EHWS), and ending some 35 m to seaward, 2 ft. or so vertically below the high-water mark of neap tides. From the foot of the upper slope there extended seaward a 'middle flat' of smooth, usually ripple-marked, sand which at about 80–90 m from EHWS began to slope more noticeably to seaward. Roughly, the start of this 'outer slope' coincided with the seaward margin of the zone of *Arenicola marina* and the beginning of the zone of *Nephthys* sp. Along the inner margin of the middle flat part of the beach, a series of shallow pools retained a layer of water even at low tide. These depressions probably resulted from the churning effect of mild surf action at high water, and the steeper upper slope to wave wash at such times. The outer slope may be the result of comparable periods of wave action at low tide. Without going into detail, it is clear from the generally sinusoidal form of the tidal curve at Millport that the upper and lower slopes must experience relatively longer periods of breaking waves than does any similar area of the middle flat, over which the tide advances and retreats more rapidly, and of course the sigmoid configuration of the beach profile, once established, further accentuates the differences in duration of wave action resulting from the character of the tidal curve. It is in the relatively stable middle flat that *Nereis* and *Arenicola* are established in large numbers. The presence of these forms has been stated by Dahl (1952) to be characteristic of mud flats rather than of sand beaches of the sort whose zonation he has recently described, and it might not be unfair to characterize this part of the Kames Bay sands as a mud flat lacking mud. In stormy weather and throughout the winter months the long wash and sweep of waves tend to fill in the angle at the foot of the upper slope. By November 1953, the foot of the upper slope had advanced about 10 m seaward from its earlier level, and by February 1954, the beach presented an uninterrupted, slightly concave, slope from the centre of the middle flat nearly to EHWS mark. The morphology of the beach is obviously not static, but represents a dynamic equilibrium of complex forces.

The substratum varies somewhat in different parts of the beach, the upper slope being of noticeably coarser sand than the middle or outer zones. However, the populated parts of the beach are, on the whole, of remarkably uniform particle size (according to Watkin, chiefly 0·2–0·5 m) with a very small fraction of silt or clay, and no black deposits in the areas studied. The most striking discrepancy in substrate occurs at the foot of the upper slope where the sand is cut away in summer to a thin layer about 3 in. deep, overlying some 3 in. of stony gravel, and this in turn covering a hard layer of mixed stones embedded in fine reddish sand.

A most noticeable feature of that section of the beach which receives no surface streams of fresh water is the fact that the lower half of the upper slope and the middle flat to seaward of its foot remain continuously wet and glistening at low tide. The appearance suggests that subsurface seepage reaches the surface of the sands above the line where the upper slope terminates, and that it permeates the sands of the middle flat for a considerable distance. This band of wetness occupies the greater part of the width of the beach. A small well-drained band occurs near the ledges bounding the beach on the east, and a wider 'dry' band is found in approximately the centre 15–20% of the beach just to the east of the main stream. The characteristic wetness of the sands in the rest of the beach seems to be present regardless of immediate weather conditions. It has been observed in the course of this study from October to April, and is evident in an aerial photograph taken in August 1947 (Fig. 2). Since this drainage appears to be of fresh water, the sands of the inner edge of the middle flat must be to some extent exposed to brackish conditions during the period of low tide. It might be assumed that any intrusion of brackish water would be replaced by sea water at high tide, but this is not necessarily so, and it appears from the literature that few workers have considered in detail the possible variations of salinity in the interstitial water that may occur on sand beaches during the tidal cycle. Bruce (1928) discusses surface and subterranean intrusion of fresh water on the beach at Port Erin and concludes from a single series of observations that '...there is a progressive fall in salinity [of the interstitial water] from the time the beach is uncovered until the advancing tide has nearly reached the point of observation, when the salinity rapidly rises to the full sea-value'. No interstitial salinities were recorded from beneath sea water, and it seems probable that Bruce was working in an area of surface drainage of fresh water. Pirrie, Bruce & Moore (1932) did not attach much importance to lowered interstitial salinity at Port Erin, for they conclude that 'no very close correlation must be looked for between the distribution of the fauna and the salinity of the beach'. Emery & Foster (1948) have shown that the salt-water table in the sands of exposed Californian beaches rises and falls with the tides, but lagging by 1–3 h and with decreasing amplitude as the land is approached. Such a fluctuating salt-water table might be of considerable importance to animals of the upper mid-tidal sands. In attempting to assess the importance of the apparent freshwater intrusion into the *Nereis* zone at Kames Bay, two possi-

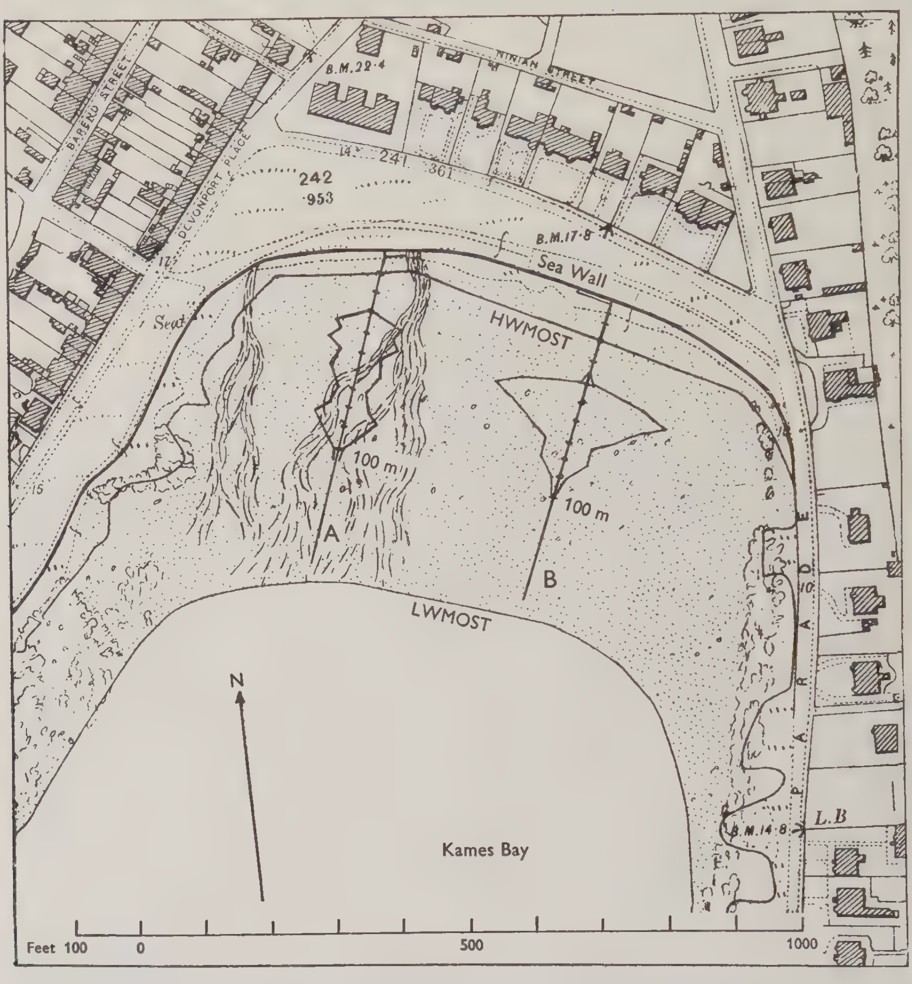
BAREND STREET
DEVONPORT PLACE
B.M.22·4
NINIAN STREET
14
241
361
242
·953
B.M.17·8
Sea Wall
Seat
HWMOST
100 m
100 m
A
B
LWMOST
PARADE
10
B.M.14·8
L.B
N
Kames Bay
Feet 100
0
500
1000

Fig. 1. Map of Kames Bay, a composite from Ordnance Survey sheets, Buteshire, CCXVI–10 & 11 and –14, 1896 edition, 1:2500; reproduced by permission of Ordnance Survey Office. Sea wall, streams, and transects A and B added. Polygons on transects show relative density of *N. diversicolor*.

bilities were considered: (1) If the interstitial drainage from the land were super-imposed upon a fluctuating salt-water base, the freshwater layer might be elevated at high tide so that the nereid zone would receive fully saline sea water twice daily. But if (2) the level of freshwater drainage were determined by geological factors which precluded oscillation of the salt-water table, then

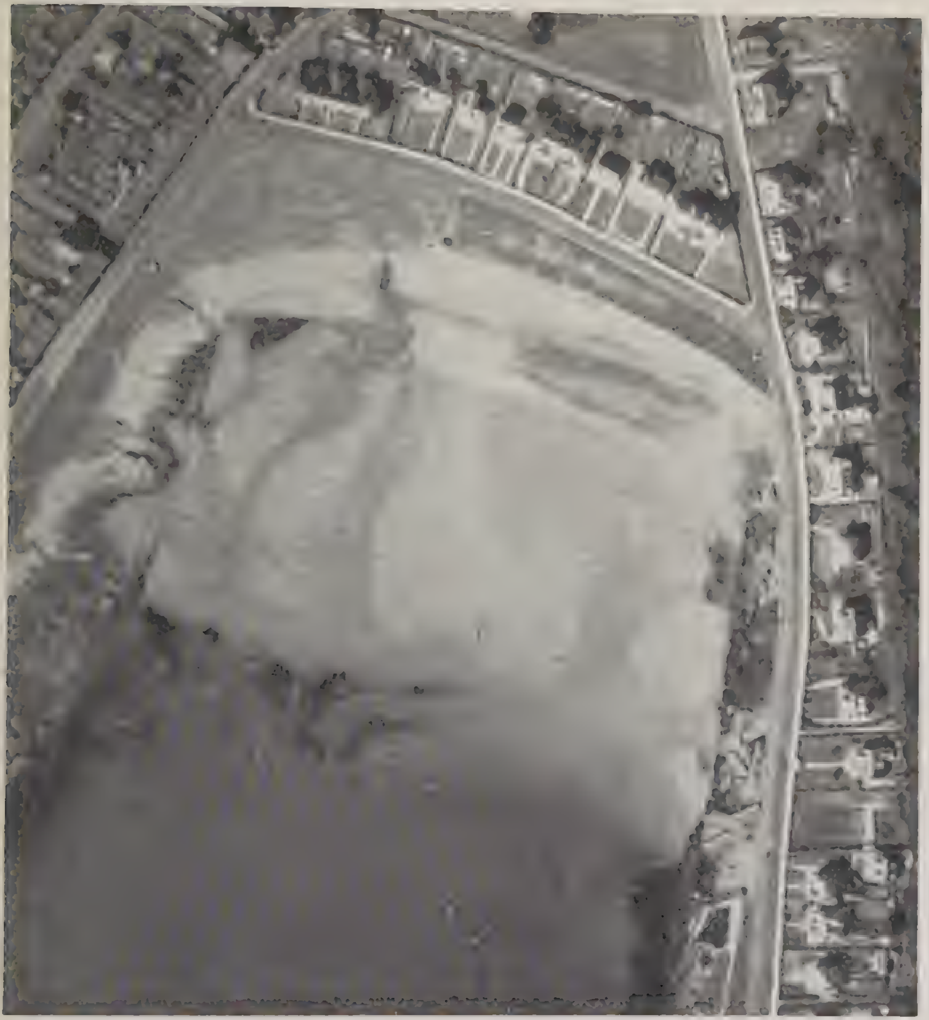

Fig. 2. Unretouched aerial photograph of Kames Bay, apparently at low tide, August 1947, showing as a darker area the position of the wet band in the upper slope of beach. (R.A.F. photo no. 4103, CPE/SCOT/UK/261, 13 August 47, F/20′/16,600′, 82 Sqdn. Crown Copyright Reserved. Published by permission of the Air Ministry.)

it would be possible to conceive of a situation where fresh water flowed at a fixed level regardless of the tide, and so penetrated the nereid zone beneath the incoming tide, subject to mixing with the overlying sea water. The latter possibility would be of obvious interest when one considers the occurrence of such a typically estuarine form as *N. diversicolor* on an apparently marine beach.

METHODS

Transects A and B are indicated in Fig. 1 and may be readily located by reference to houses near the bay. Transect B, on which most work was done, started at the lower eastern corner of the sloping concrete apron at approximately EHWS mark and ran directly seaward. Distances were measured with a 25 m length of trawl line marked off in 1 and 5 m intervals. Although subject to error by stretching, it gave sufficiently reproducible results for the purpose. Water samples were sucked up from a depth of 6 in. beneath the sand surface by means of a rubber tube and a stiff plunger, originally of Pyrex tubing (of 5 mm bore, flamed down at tip to 1 mm), later replaced by a brass tube of comparable dimensions. It was found best to make the orifice large enough to admit sand grains freely; these tended to settle and did not seriously interfere with sampling. For use in coarse sand the orifice is better placed at the side of the tip rather than terminally. In use, the plunger was pushed to the desired depth (a large cork on the tube formed a stop), a sample sucked up and discarded, the tube re-inserted while blowing to exclude water, a second sample brought up, the tube wiped off, a small quantity of water discharged, and the remainder collected in a vial. When subsurface samples are taken in this simple way from beneath overlying sea water there is likelihood of contamination, but as such would only tend to lessen the differences observed, the general conclusions reached would not be invalidated. Aliquots of 1 ml. were titrated from a 5 ml. burette with $AgNO_3$, 19·16 g/l., equivalent to 4 g chloride per litre, standardized against a carefully prepared solution of NaCl (equivalent to 10 g chloride per litre), diluting each aliquot with 10 ml. of distilled water and using 5% potassium chromate indicator. Since this procedure is not that of standard hydrographic practice, the results have been expressed in grams of chloride per litre at room temperature (a value approximating 'chlorosity') rather than as 'chlorinity'. The differences, for our purpose, are inconsequential. For obtaining the density of worms at each station a square box of galvanized sheet-iron without bottom, of $\frac{1}{10}$ m^2 area, was pressed into the sand to a depth of 6 in., the enclosed sand dug out, and washed through a sieve of 16 meshes to the inch. Only *N. diversicolor* were collected; counts are expressed in numbers of heads obtained regardless of size, although it was noted that the larger worms were more abundant at about the 50–60 m level. Transect B was studied on 31 October–2 November 1953, 12–13 February, and 18 March 1954, with special attention paid to obtaining interstitial water samples from beneath overlying sea water at high tide or during the ebb, when salinities in the sand might be expected to be maximal. Unpublished rainfall and salinity data recorded at the Millport Laboratory were kindly made available by Dr H. Barnes.

OBSERVATIONS (TRANSECT B)

31 *October–2 November* 1953. Two series of water samples were taken on falling tides and one on a rising tide; one series of collections of *N. diversicolor* was taken at corresponding stations. On 1 November the transect was sampled successively 5 times, from 1 h past the start of the ebb until the turn of the tide. On this day the foot of the upper slope lay at 50 m from EHWS mark, having advanced somewhat from the mid-October position, but the break at its foot, where the stony layer lay close beneath the surface, was still distinct. It was raining intermittently, following a fortnight of heavy rains (the Marine Station had recorded 5·39 in. of rain in the previous 15 days). The interstitial chloride profiles (Fig. 3 A–F) indicated a brackish zone which tended to move seaward as the tide fell, and which was distinct even beneath the shallow wash of the retreating sea water. As the tide fell, the surface water became progressively less saline, probably as the combined result of dilution by subsurface drainage and of lateral mixing with fresh water from the streams to the west. Under the conditions of high freshwater discharge prevailing that day the chloride of the bay water was lowered from 17 to 12·8 g/l., but at the turn of the tide the inflooding sea water had a nearly normal chloride value of 17 g/l. (=salinity of 30·5‰).

On 2 November, two series of interstitial water samples taken beneath sea water up to 20 in. deep on the falling tide confirmed the results of the previous day and showed more clearly the low salinity of the interstitial water beneath ebbing sea water (Fig. 3 G, H). The lowering of subterranean chloride was apparent out to 50–60 m from EHWS mark. The possibility was considered that these results reflected abnormal conditions caused by heavy rainfall in the previous 2 weeks.

12–13 *February* 1954. The earlier work was repeated following a period of relatively dry weather. There had been rains on 5–6 and 9–10 February, but the total rainfall for the previous 17 days had been only 1·57 in. By this time winter storms had so smoothed out the upper half of the beach that no break was apparent between upper slope and middle flat. Several inches of sand had been deposited above old burrows of *N. diversicolor*, but with little or no displacement of the population. The zone of *Arenicola* was unaltered. One salinity profile was taken during rain on 12 February on a rising tide and a second series of three in light rain on the ebbing tide, 13 February. The chlorides obtained on the falling tide (Fig. 4) agreed with the previous findings, brackish interstitial water being evident beneath sea water and detectable out to 60–70 m, about to the inner edge of the zone of *Arenicola*.

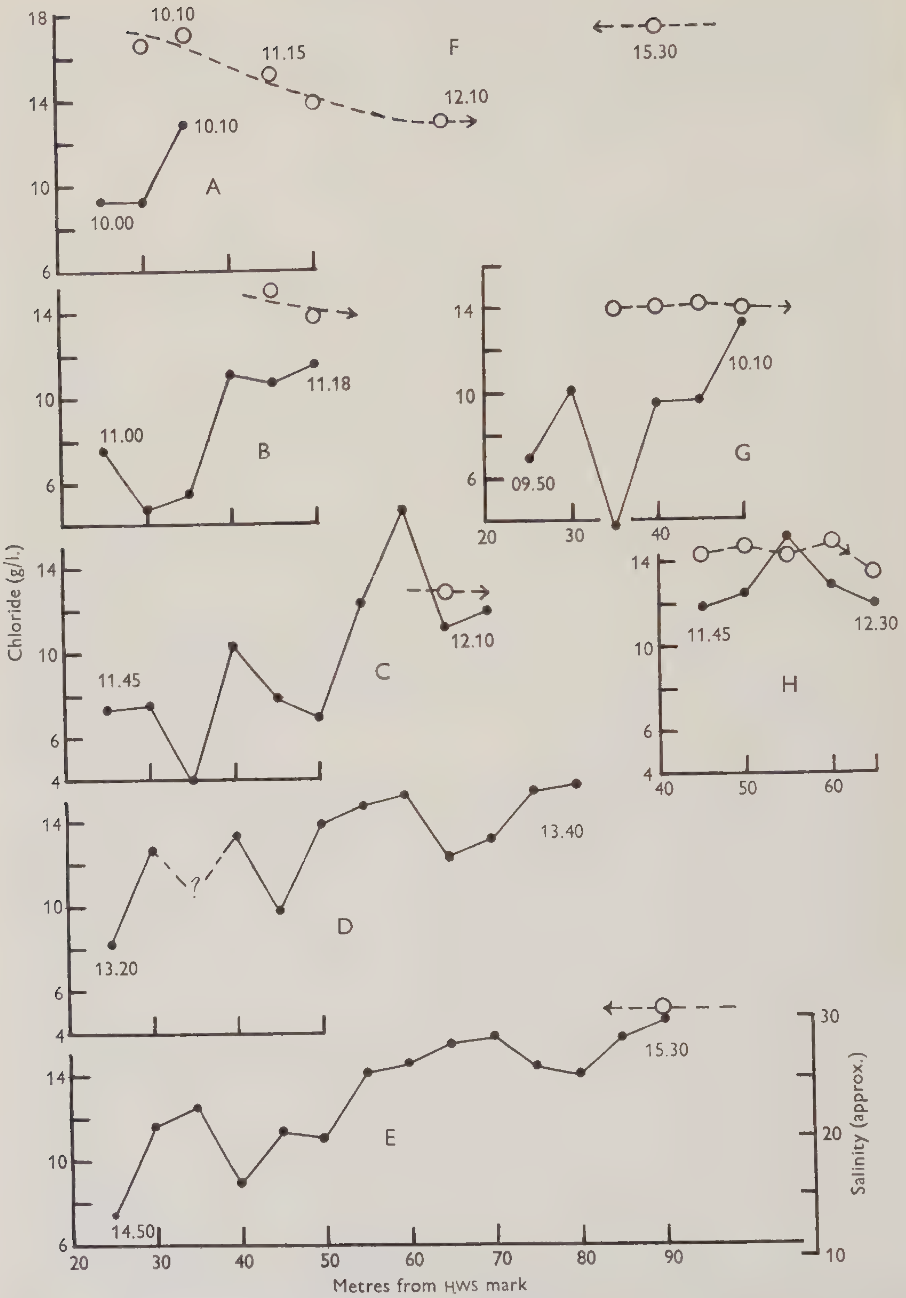

Fig. 3. 1–2 November 1953. Solid curves A–E, chloride of interstitial water on falling tide 1 November, taken successively at times indicated; dashed curves, chloride of overlying sea water, plotted collectively as curve F, the point at 15.30 being at start of rising tide. Insets G and H, similar, for falling tide on 2 November.

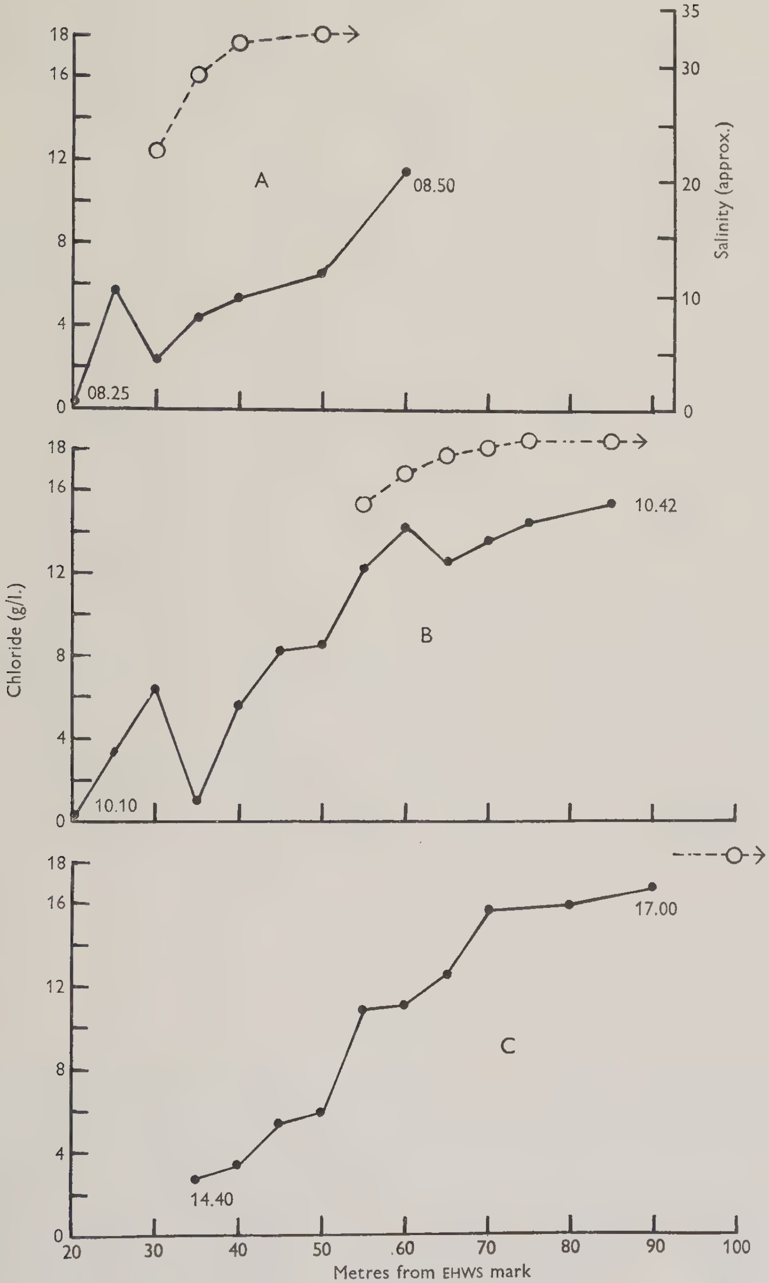

Fig. 4. 13 February 1954, falling tide. Solid curves, interstitial chloride; dashed curves, chloride of overlying sea water. Times of sampling as indicated.

Some dilution of the sea water by land drainage was noticeable at the start of the ebb. It would appear that the presence of brackish water beneath sea water was unaffected either by the winter changes in beach configuration or by the period of relatively low rainfall. It seems unlikely that rain actually falling at the time of sampling could have produced these results.

The distribution of *Nereis diversicolor* as found on the two occasions discussed above is shown in Fig. 5A, B. The population is sharply limited on its shoreward edge, reaches its maximum at about 50–55 m from EHWS mark (i.e. on the inner margin of the middle flat as this is seen in the summer months), and drops markedly at about the inner margin of the *Arenicola* zone, although present in lesser numbers to about HWN level. The zonation agrees well with that reported by Watkin; his distribution of *N. diversicolor* is shown for comparison in Fig. 5, where his values are replotted on a metric scale.

18 *March* 1954. Samples of interstitial water were taken from a boat at high water. Conditions were calm and clear; there had been no rain for the previous 9 days, and a total of only 0·97 in. for the previous 16 days. For the previous 30 days the rainfall had amounted to 3·50 in., which is close to the median value of monthly rainfall over the past 5 years, and below the average monthly value of 3·67 in. for the years 1949–53. The tide was above average height, calculated from Admiralty Tide Tables as 9·7 ft. This is not an extremely high tide (the highest listed for the first 6 months of 1954 was 11·5 ft.), but the height of tide on 18 March equalled or exceeded 71 % of the 303 high tides in the above 6-month period. Although combinations of higher tides and lower rainfall may occasionally occur, the conditions seemed quite typical, and the results not attributable to exceptional weather or tidal conditions. The chloride of the sea water was 18·05 g/l. (average of three samples); that of the interstitial water (average of four samples at each station):

Station (m.)	Depth of sea water (in.)	Interstitial chloride (g/l.)
45	22	9·14
50	24	6·85
55	30	9·26

DISCUSSION

The above results indicate that on this particular beach brackish interstitial water is a characteristic and relatively stable feature of the zone of *Nereis diversicolor*. That the degree of brackishness may vary with season, rainfall, and tide cannot be denied, but it is probably to be regarded as a permanent feature of the Kames Bay sands. There is no evidence that the intrusive fresh-water drainage rests upon a fluctuating salt-water table, but rather that geological features permit it to flow into the sands of the beach with sufficient head to maintain brackish conditions in the sand even at high tide. Such conditions may be peculiar to this beach and are not necessarily to be found

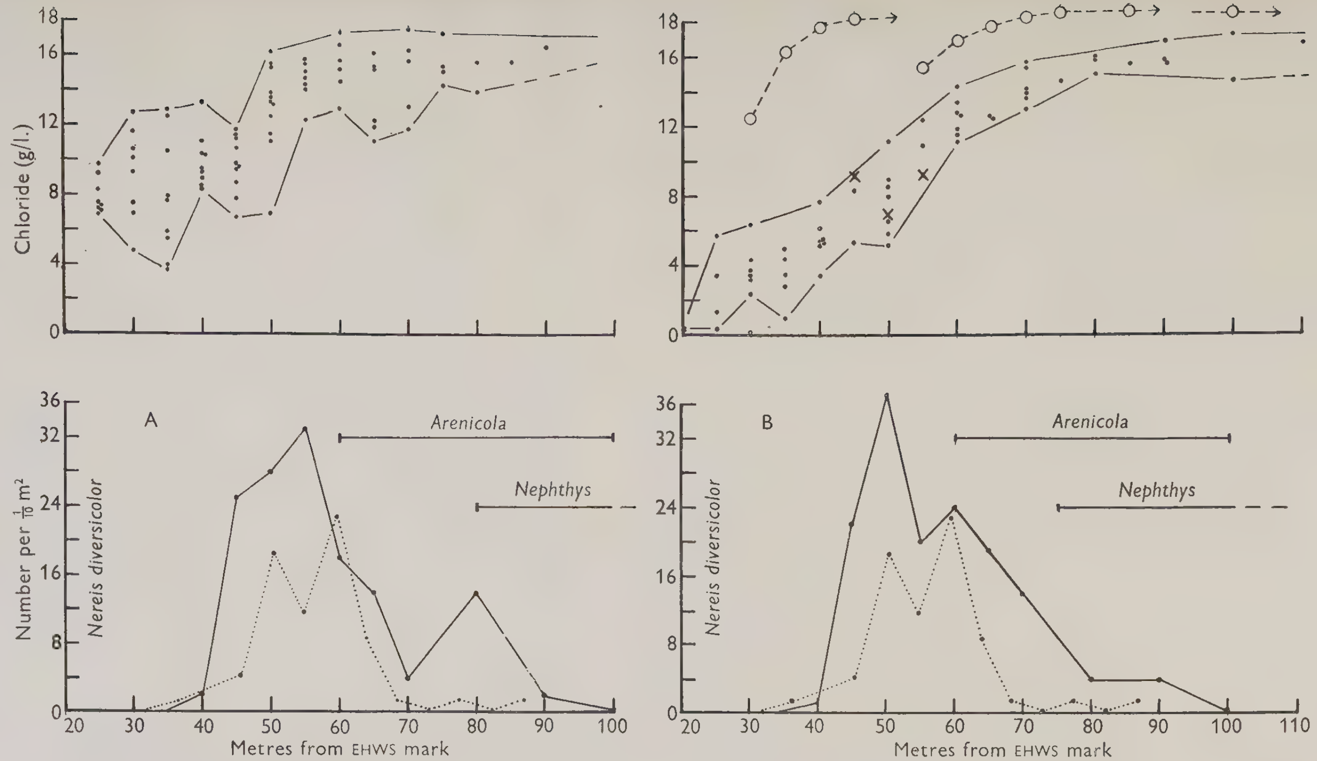

Fig. 5. A (upper), distribution of interstitial chlorides on one rising and two falling tides, 31 October–2 November 1953; B (upper), same for one rising and one falling tide, 12–13 February 1954, plus chlorides of overlying seawater during ebb 13 February and interstitial chloride (×) at high tide 18 March. A, B (lower), distribution of *N. diversicolor* in present studies (solid curves) in worms per 0·1 m^2. Dotted curves show distribution of *N. diversicolor* as reported by Watkin (1942), replotted on metric scale. Horizontal bars indicate range only of certain other polychaetes as noted in present study.

on other beaches. That they may be more common than hitherto noted is suggested by the fact that interstitial salinity lower than that of sea water has been observed beneath the ebbing tide at Fintray Bay, Isle of Cumbrae, where the beach is totally unlike that at Kames Bay, being exposed, steeply sloped, and of very coarse, loose, gravelly sand and pebbles.

A full understanding of the course of freshwater drainage into the Kames Bay sands would require more geological evidence than is at present available, and it is not possible to attribute the course of drainage into the beach to any particular geological configuration or structure. The bay includes the Great Cumbrae Fault 'to which we owe the straight eastern boundary...' (Gunn, 1903). This fault causes the bay to be bounded on the east by Upper Old Red Sandstone (Devonian) but on the west by Basal Carboniferous Calciferous Sandstone. The latter rocks are reported to dip southward toward Kames Bay and to include red clays or shales. If the actual fault lies beneath the eastern side of the bay, then the Carboniferous strata may underly the site of this study, and might be considered to facilitate drainage into the sands, but proof is lacking. A large but inconspicuous double dike cuts through the rocks at the eastern shore of the bay, although its termination beneath the sands at the actual fault has never been located. On the present evidence no assessment of the geological factors in the situation can be made; more studies on a variety of beaches are needed. I am indebted to Mr T. B. Bagenal of the Millport Laboratory and Mr W. G. E. Caldwell of Millport for discussions of the local geology.

The question whether to regard the concentration of *N. diversicolor* as an instance of intertidal zonation or as an example of a normally estuarine species being localized in an area of optimal salinity is not easily answered. There is no doubt that individuals of the species can survive in pure sea water. Possibly the lesser number found beyond the 60 m level at Kames Bay simply results from 'competition' by *Arenicola* and several other species unable to populate the zone of low salinity in which *N. diversicolor* thrives. The zonation of *N. diversicolor* may reasonably be related to brackish interstitial water, but it is not clear whether the brackish conditions *per se* are favourable, or whether these conditions serve to limit competition, or whether the zonation observed is in part a proper 'intertidal' zonation dependent upon tidal factors exclusive of salinity.

From the point of view of the physiology of *N. diversicolor* itself, it is of interest to determine what changes of salinity it must encounter in its zone of maximal abundance. In this connexion, extremes of salinity and rates of salinity change are more critical than mean salinity. In order to examine this point, all the interstitial chloride values obtained on 31 October–2 November 1953 (both on rising and falling tides) have been plotted in Fig. 5A, and all such values for 12–13 February and 18 March 1954 in Fig. 5B. From these charts, in which the extremes of chlorinity are outlined, it would appear that whereas *Arenicola marina* must seldom encounter chlorinities below 12 parts per mille in Kames Bay, the bulk of the nereid population may regularly in the course of a day have to endure chlorinities between 12 and 5–6‰, and some must experience chlorinities as low as 4‰. That this is well within the tolerance of the population is suggested by laboratory experiments in which worms from Kames Bay have been adapted by steps to chlorinities as low as 0·25‰. This is about 1·4% of Millport sea water, which has a mean chlorinity of 17·76‰ (=salinity 32·90‰), with monthly chlorinity means varying from 18·43 to 16·55‰ (based upon 5-year period June 1948 to May 1953).

If the field observations reported here may be regarded as of typical conditions, *Nereis diversicolor* is not exposed at Kames Bay to salinities which in themselves approach the limit of its osmotic tolerance. It is likely that at high tide the worms experience salinities above the values observed for the interstitial water, since these worms probably resume irrigation (and may emerge to feed) as soon as the rising sea water covers them (Wells & Dales, 1951). Hence, while the actual salinity endured at low tide may be that of the interstitial water, the salinities experienced at high tide must be the resultant of circulated sea water being diluted by brackish water in the sand about the burrows. The fact that brackish water remains available may well protect the worms against too abrupt rises of salinity at each high tide, and thus lessen the impact of salinity changes, somewhat as does the salt-water content of estuarine flats exposed to over-flowing fresh water at low tide. The actual rate of change of salinity experienced by *N. diversicolor* at Kames Bay cannot be determined from the present data, but an inspection of Fig. 5 suggests that in the zone of maximal abundance the worms experience a change in chlorinity of not less than 6 parts per mille per tidal rise or fall, and probably within 2 h.

These studies were carried out while the author held a Fulbright Exchange Lectureship in the Zoology Department, University of Glasgow. Field work and analyses were done at the Scottish Marine Biological Association Laboratory at Millport, to whose Director and staff I am grateful for much kindness and assistance.

SUMMARY

An hitherto undescribed, relatively stable, zone of brackish interstitial water is reported from the upper mid-tidal sands of Kames Bay, Millport.

Lowered interstitial salinity persists even beneath overlying sea water at high tide. Present evidence does not permit evaluation of the geological factors involved.

The zonation of *Nereis diversicolor* on this beach seems correlated with the belt of lowered salinity, but it remains unclear whether the brackish conditions *per se* are favourable, or if they serve to limit competition from other species.

It would appear that the observed zonation is not purely dependent upon tidal factors exclusive of salinity.

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INNERVATION OF THE HEART OF *PRAUNUS FLEXUOSUS* (MYSIDACEA)

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(With Plate I and Text-fig. 1)

The heart nerves of *Praunus flexuosus* Leach (= *Macromysis flexuosa* White) have been stained with methylene blue applied in various ways. As a rule the animals were injected with a solution prepared immediately before use by mixing 1 part of 0·5% solution of methylene blue in distilled water or of rongalit white with two parts of sea water. The injection was made into one of the posterior segments of the abdomen with the needle pointing forwards. When three or four animals are treated at the same time, usually in at least one of them the staining reaction takes a satisfactory course and its progress can be observed under the microscope, owing to the transparency of the tissues. In a few minutes after the injection some of the nerves are already visible. Later the coloration becomes more general and more intensive, but soon begins to fade out and can disappear completely.

For staining the heart nerves by a second procedure, i.e. by immersion in a weak solution of the dye, the animals should be sectioned along the ventral side and their digestive organs and gonads taken out. It is, moreover, necessary to remove the large chromatophores situated on the ventral side of the pericardial diaphragm, and this membrane has also to be pulled out. The heart thus becomes exposed with its ventral wall uppermost, which can be cut along the middle line to give better access to the main trunks of the heart nerves situated on its dorsal side. The same way of exposing the heart has to be followed when the preparations obtained by the method of injection are to be fixed, as the ammonium molybdate solution does not penetrate through the chitin.

The fixation of the staining caused the same difficulties as experienced with amphipods (Alexandrowicz, 1954). It is obvious that in these animals the bond between the dye and the stainable substance of nerves is of a very unstable nature, and only at the moment when the coloration attains the peak of its intensity the fixation may give somewhat more satisfactory results. It is difficult to estimate this right moment, especially as various nerve elements may take up the dye at different times. Thus, for instance, the nerve cells in the heart of *Praunus* stain but rarely and always later than the fibres. By waiting too long for their possible appearance many otherwise good preparations are lost.

OBSERVATIONS

The heart of Mysidacea situated in the thoracic region has a shape of a fusiform tube continuing forwards and backwards into the anterior and posterior aorta respectively, the only vessels which can be seen from the dorsal side (Text-fig. 1 A). As stated by Delage (1883) the limits of the heart tube, which at its ends does not differ in width from these vessels, can be determined by the presence of the valves. It may be added that the muscle fibres of the heart do not pass on to the arteries and therefore the places where they stop mark the boundaries of the heart. The muscle fibres, of finely cross-striated type, are arranged in a single layer. They turn in right-handed spirals as in the Isopoda, whereas in the Amphipoda the direction is reversed.

From the ventral side of the heart arise several arteries. The largest of them, the sternal artery, originates at a certain distance from the posterior aorta and a little to the left of the middle line. The two hepatic arteries arise close to each other and to the anterior aorta. According to Delage there are, in addition, two unpaired arteries originating between the hepatic and the sternal arteries: the anterior of them, which as this writer remarks is very slender, could not be recognized in methylene-blue preparations.

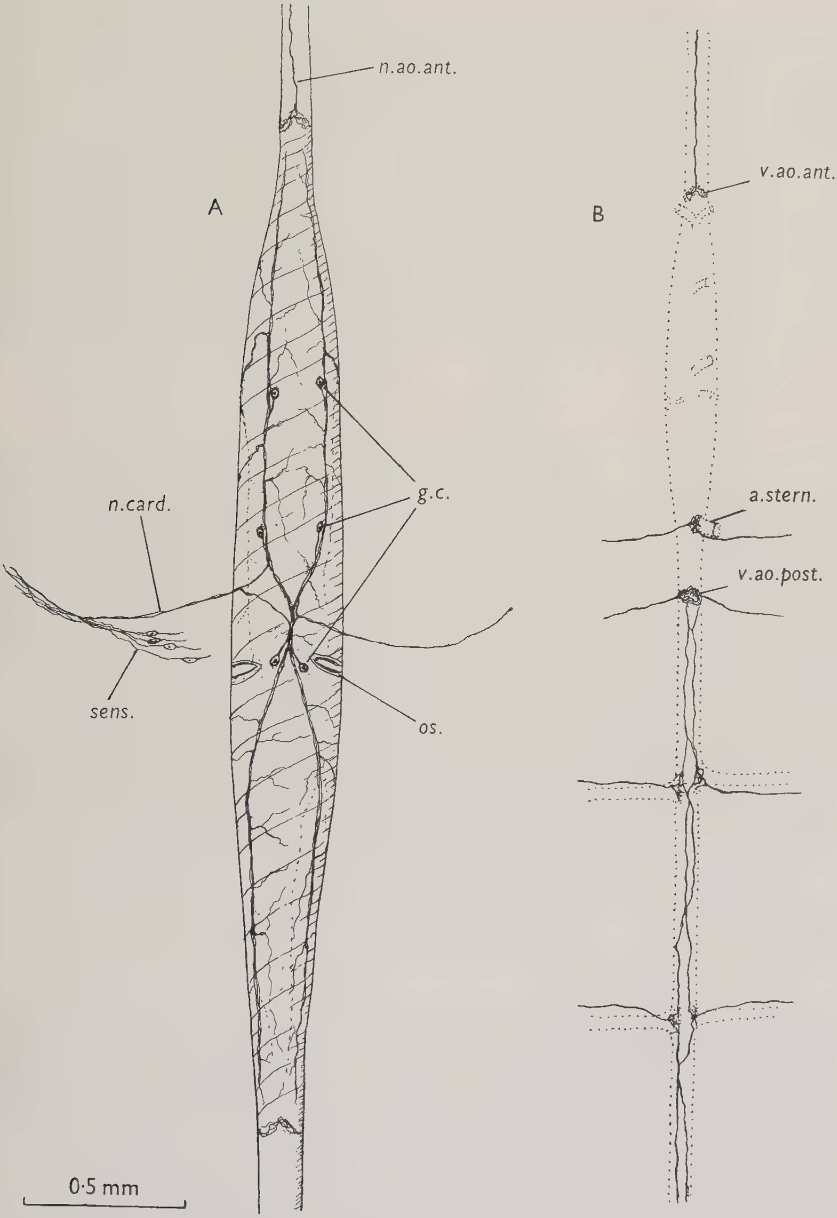
Nerves of the Heart

The three systems of nerves observed in the heart of all Malacostraca investigated up to now have been also found in the Mysidacea. There are: (1) a local system made up of neurons situated in the heart itself, (2) nerves connecting the local system with the central nervous system, and (3) nerves of the arterial valves.

Local System

The main elements of the local system are situated on the outside of the dorsal heart wall. A short trunk, lying just in front of the ostia and bifurcating at each end, forms a characteristic figure with the branches resulting from these divisions (Text-fig. 1 A; Pl. 1, fig. 1). The two anterior and the two posterior trunks, running in opposite directions, and traceable up to the ends of the heart, give off many branches, the largest of them passing on to the ventral side of the heart.

The ganglion cells, the axons of which make up these trunks, number no less than six. Two of them are situated near the posterior end of the unpaired main trunk, and four on the anterior trunks, two on each of them (Text-fig. 1 A, *g.c.*; cf. the photograph, Pl. 1, fig. 3, on which four of the cells are seen). Their situation can vary; the middle pair can lie nearer or farther from the main trunk, but the most anterior ones have always been found far forwards approximately midway between the ostia and the anterior end of the heart. The cells usually look as if they were unipolar; only rarely some dendritic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A
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Text-fig. 1, A: *Praunus flexuosus.* View of the heart from the dorsal side showing the main nerve trunks and their branches. *g.c.*, ganglion cells; *n.card.*, cardiac nerve; *sens.*, sensory nerve cells; *n.ao.ant.*, nerve of the valve of the anterior aorta; *os.*, ostium. B: nervous system of the arterial valves. The heart is seen from the ventral side. *v.ao.ant.*, valve of the anterior aorta; *v.ao.post.*, valve of the posterior aorta; *a.stern.*, sternal artery.

processes are distinguishable (Pl. 1, figs. 4, 5). It seems, however, more likely that they are multipolar, but that the processes do not stain clearly. The nucleus of the cells is of conspicuous size, markedly larger than in the ganglion cells of the heart of other crustaceans.

The axons of the four anterior cells are directed backwards, and those of the two posterior cells forwards. This does not imply that all their further ramifications run in the same way. The area of the heart supplied by individual neurons could not be determined, but from the course of the branches given off by the main trunks and running far in both directions it may be inferred that each part of the heart is supplied by several neurons. As usual in the Crustacea, the more successful preparations show a remarkable richness of fine nerve filaments supplying the muscle fibres.

Nerves Connecting the Local System with the Central Nervous System

The paired nerve (n. cardiacus) coming from the central nervous system approaches the heart approximately in its mid-length (Text-fig. 1 A; Pl. 1, fig. 2, *n.card.*). It is seen here in association with the nerve that carries fibres of the sensory cells of the middle region of the carapace (Text-fig. 1 A, *sens.*), but whether it travels with the same nerve from the ventral ganglionic cord is not certain. There has been even some evidence that it follows a different route; however, because of technical difficulties, no clear picture of the proximal course of the nerves in this region could be obtained. Passing on to the dorsal side of the heart the cardiac nerve joins either the anterior trunks (Pl. 1, fig. 2) or the main trunk or, not uncommonly, the points of junction are different on opposite sides (Text-fig. 1 A). Entering the trunk, or even before doing so, each nerve bifurcates, sending branches in opposite directions. It has not been possible to trace their further course. Neither am I able to state the exact number of fibres conveyed by these nerves from the neural cord. Two of them are often met with, but in view of the fact that the finer fibres may not stain at all this observation is not conclusive.

Nerves of the Arterial Valves

The nerve supplying the valve of the anterior aorta runs down this vessel in the same way as does the nerve of Lemoine in the Decapoda. In animals injected with the methylene blue it stains in a few minutes and can be seen by transparency in its course on the dorsal surface of the stomach up to the fine filaments terminating on the valve which may still be active.

The nerves to the valves of the posterior aorta and sternal artery are given off by the segmental nerves running on the ventral side of the extensor muscles and therefore can be visible only after dissection of the animals. They do not stain so readily as in the anterior aorta and can be but rarely noticed, the more so that during various manipulations described above the fine nerves can be easily torn away. Maybe for the same reason I was unable to trace the nerves to the remaining four arteries arising on the ventral side of the heart. On the other hand, it has been easy to observe the innervation of the posterior aorta in its course down the abdomen. This vessel, called also abdominal artery, is supplied in each segment by fine nerves running along its segmental branches. These nerves give off two sorts of fibres: some, very short, end at the origin of the segmental paired vessels; others run alongside the abdominal artery establishing connexions of the whole system including the valve at the posterior end of the heart. The arrangement is strikingly similar to that found in decapods (Alexandrowicz, 1929, 1932), and it seems highly probable that this sytem of nerves in mysids is also destined to supply the valves at the exit points of the segmental arteries in the abdomen, although the latter could not be clearly seen in *Praunus*.

DISCUSSION

The recorded results provide another proof, that, in the Crustacea, various components of the heart innervation follow one fundamental pattern. The differences observed appear to be rather of minor character. As regards the local system in mysids its peculiar feature, compared with amphipods and isopods, is the arrangement in pairs of the ganglion cells and their situation at a certain distance from the middle line, whereas in the two other groups all cells lie in the unpaired median trunk. The number of cells (six) found in *Praunus* is the same as stated for *Ligia oceanica* (Alexandrowicz, 1952) and assumed to be the most probable in *Marinogammarus marinus* (Alexandrowicz, 1954). However, rather than to admit that this coincidence be not a fortuitous one, I am bound to express doubts whether the number found in *Praunus* is correct. For one reason, it is surprising that the posterior part of the heart, about half of its total length, should be deprived of nerve cells. In various crustaceans with an elongated heart such as the Amphipoda, Isopoda and Stomatopoda, ganglion cells are present along the whole of its length, and in the Decapoda it is in the posterior half that the ganglionic trunk, or the major part of it, is located. Also, in view of the symmetrical disposition of the main trunks in mysids, the presence of the cells in the posterior trunks could be expected. The negative results may be due to the insufficient staining of cells in this region. Moreover, owing to the deeper situation of the posterior part of the heart, it is much less accessible to observation by transparency which has been otherwise very helpful.

As regards the nerves connecting the local system with the central nervous system it must be pointed out that only one pair of them has been noticed in mysids, whereas in amphipods two such paired nerves and in stomatopods even three are present. Perhaps in mysids as in decapods the cardiac nerve conveys fibres of different kind and origin, but, as mentioned before, its proximal course could not be determined. On the other hand, it is not certain whether there is really only one pair of the cardiac nerves. With animals of such a small size nerves made up of very tiny fibres may not show at all.

It is possible for this reason that I could not detect the nerves on the pericardial diaphragm which, as in other crustaceans, might be expected to supply the muscle fibres of the pericardium. It is true that such muscle fibres were not observed, but this again may be due to the short-comings of the technical methods.

Uncertainty also remains regarding the occurrence of the pericardial organs, i.e. nerves of special structure which have been found in the Decapoda, Stomatopoda and Amphipoda. In *Praunus* there are nerve elements which in their arch-like arrangement resemble the pericardial organs in *Leander serratus* (see Alexandrowicz, 1953). However, because of their insufficient staining and even more because of their small dimensions, their nature could not be established. It is to be hoped that with species of larger size this point, as well as others mentioned above, might be satisfactorily elucidated.

Summary

In the heart of *Praunus flexuosus* three systems of nerve elements have been found. The local system consists of no less than six neurons. Their cell-bodies are situated on the dorsal heart wall near the short unpaired trunk and its two anterior branches. The axons of the nerve cells innervate the muscle fibres of the heart. A pair of cardiac nerves joining the trunks of the local system establish the connexion with the central nervous system. A separate set of nerves supplies the valves of the vessels arising from the heart. The nerves of the valve of the posterior aorta are connected with the nerves running alongside this vessel which receive segmental branches coming from the abdominal ganglia.

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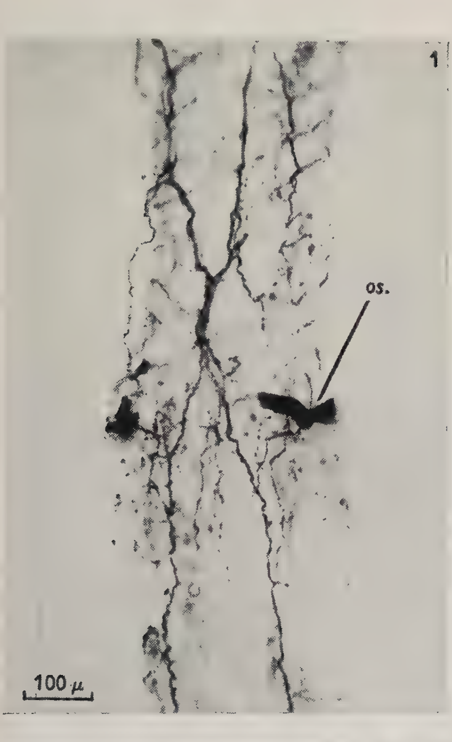
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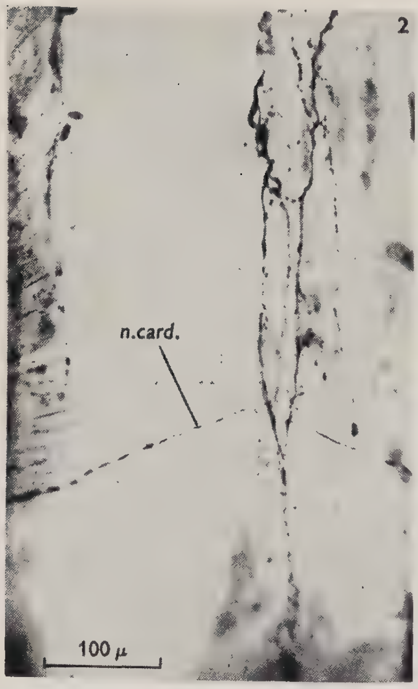
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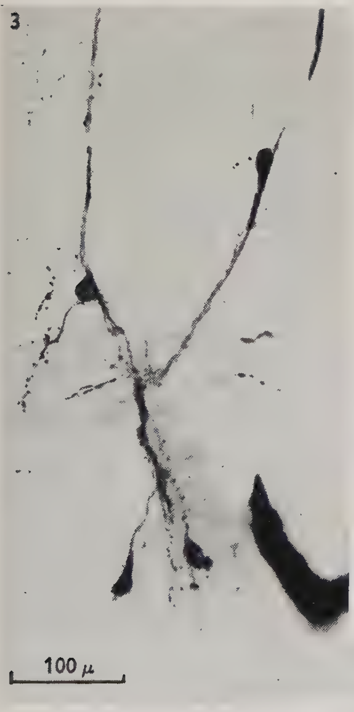
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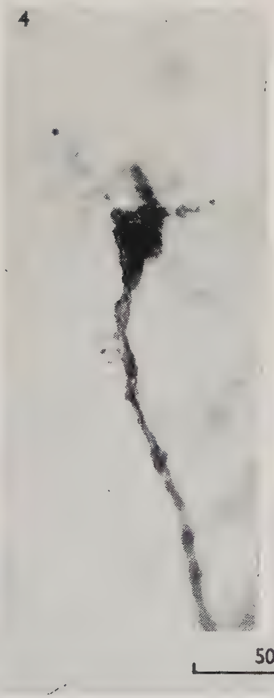
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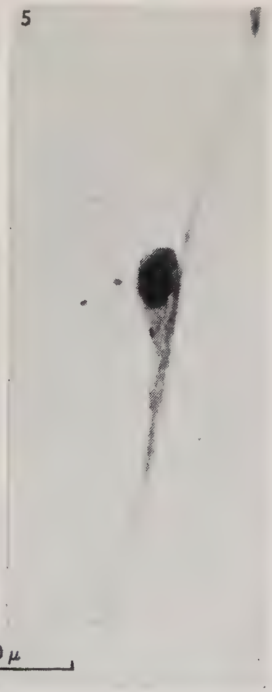
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EXPLANATION OF PLATE I

All photomicrographs have been made from preparations of the heart of *Praunus flexuosus* stained with methylene blue, fixed in ammonium molybdate and mounted in dammar-xylol.

Fig. 1. Middle part of the heart with main nerve trunks and some of their branches. Ganglion cells and cardiac nerves are not visible. *os.*, ostium.

Fig. 2. Middle part of the heart with main trunks and cardiac nerves (*n.card*) entering the anterior trunks. The position of the anterior trunks close to each other is due to elongation of the heart produced artificially.

Fig. 3. Middle and posterior pair of ganglion cells.

Fig. 4. Anterior ganglion cell.

Fig. 5. Middle ganglion cell. Note the size of the nucleus.

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FEEDING AND DIGESTION IN TEREBELLID POLYCHAETES

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(Text-figs. 1–9)

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Introduction

Most of the naturalists of the nineteenth century were attracted by the tube-building abilities of the terebellid polychaetes rather than by their feeding behaviour. As Dalyell (1853) says in his *Powers of the Creator*, referring to terebellids: ‘All are architects, nor of contemptible skill, as we shall find on contemplating their mechanical labours.’ Perhaps the best known English work dealing with this subject is the paper by Arnold Watson (1890) on the tube-building habits of the sand mason (*Lanice conchilega*), and while concerned mainly with tube building, the paper does include some observations on the feeding habits. Earlier, Gosse (1854) had recorded his observations on the behaviour of terebellids, and some additional information may be found in Quatrefages's Treatise of 1865. The first serious attempt to find out more about the morphology and histology of the gut in terebellids was made by Claparède (1873), and this work was published posthumously in his monograph on the Polychaeta Sedentaria. Steen (1883) soon after recognized the division of the terebellid stomach into glandular and muscular portions, and this morphological work was further extended by Meyer (1887) in his ‘Studien über den Körperbau der Anneliden’, and, more especially, by the Swedish morphologist Axel Wirén (1885). This latter paper forms the most substantial contribution to our knowledge of the anatomy of terebellids. Work on this group at Uppsala was extended further by Hessle (1917, 1925). Some information may also be found in McIntosh's (1922) Monograph, and in the Memoir on *Amphitrite* by Thomas (1940), but few details are given about feeding and digestion in the latter work, and most of the descriptions in the former are misleading.

Feeding and digestion have been studied in only a few polychaetes. The most important paper is that of Brasil (1904) on *Pectinaria koreni*, and this includes a useful résumé of the previous work on feeding and digestion in polychaetes. The paper by Nicol (1931) on *Sabella* is perhaps the best-known work published since then, and additional references to gut morphology in polychaetes will be found also in the recent paper by Ullman & Bookhout (1949) on the histology of the gut of the maldanid, *Clymenella*.

An attempt has been made in the present paper to give a comparative account of the feeding mechanism and the functional morphology of the gut in the principal genera of the Terebellidae. Most of this work has been done at Plymouth, but observations have been made elsewhere, both in this country and in America, on the feeding habits and the structure of the organs concerned. Although Thomas (1940) has given a useful account of some aspects of the anatomy of *Amphitrite johnstoni*, the description of the gut is incomplete and may well be amplified. Owing to its large size *A. johnstoni* is the most suitable species available in this country for such studies. A description of the anatomy and feeding behaviour in this species will be presented first. Feeding and morphology of the gut will then be compared in other terebellids that have been studied.

I am indebted to the Director and Staff of the Plymouth Laboratory for their kindness and help, and to Mr Norman Tebble of the British Museum (Natural History) for supplying me with specimens of some genera not represented in our fauna, and which I have not yet had the opportunity of studying alive.

FEEDING AND FUNCTIONAL MORPHOLOGY OF THE GUT IN *AMPHITRITE JOHNSTONI*

A. johnstoni Malmgren is one of the largest of the British terebellids, often attaining a length of 20–25 cm and a breadth of 2 cm or more in the anterior region of the body. It is a pink or brown worm living in mud or very muddy sand, and in spite of Dalyell's eloquence, it does not construct a tube. The mainly vertical burrow may be 2 or 3 times the length of the worm and fundamentally is always U-shaped. There is no distinction between the two openings of the burrow as the worm can, and frequently does, reverse within the burrow. Near Plymouth this species can be obtained in numbers only at low-water mark of spring tides in the River Yealm and at Salcombe.

As in all terebellids the prostomium is represented externally by a large number of extensile tentacles and a cowl-like flap forming an upper lip to the mouth (Fig. 1, *Ul*). The first segment is reduced, save for the ventral part which contributes to the series of ventral lips to be described (Fig. 1, *Ol*). The anterior part of the body is much swollen, and the ventral part of each segment in this region contains well-developed mucous glands (Fig. 1, *Mg*). In tube-building species these glands are important in secreting the lining of the tube. This anterior region containing these mucous glands which open ventrally on conspicuous 'ventral shields' is often referred to as the 'thorax'. In *A. johnstoni* there is, just posterior to the thorax, a smooth, slightly swollen region of the body, and it is this region which contains the coiled fore intestine (Fig. 1, *F.int*). This is exceptional; in most terebellids the body tapers uniformly towards the posterior end, and in many there is no real division of the body into an anterior thoracic region and a posterior abdominal region. Anteriorly there are three pairs of branched gills (Fig. 1, *Br*). While respiratory exchange takes place over the whole surface of the body, these gills are obviously concerned solely with respiration. The prostomial tentacles, on the other hand, are concerned only with the collection of particles of detritus or other matter for food.

Behaviour

The feeding behaviour of *A. johnstoni* was studied in the laboratory. Worms will accept glass U-tubes of the right calibre, and will live in them for at least 6 months when placed on the bottom of a tank through which a flow of sea water is maintained. Without an artificial tube, and without adequate circulation, this species will not survive long in laboratory conditions. Worms were also studied in artificial sand and mud burrows made between sheets of glass separated by a loop of plasticine and clamped together vertically in a deep tank. Though such an arrangement simulates the natural conditions more closely than glass U-tubes lying horizontally on the bottom, it was found that the behaviour of the worms in either apparatus was not noticeably different, and most of the observations have been made on worms housed in glass tubes. Mud sandwiches have the disadvantage, not only of the difficulty of maintaining the mud in a healthy condition, but also in that the surface over which the tentacles can extend for feeding is more restricted than when the mouth of the tube lies on the bottom of a tank. The activity of the worms in both types of apparatus has been followed, and the activity recorded kymographically for continuous periods of up to a week for any one worm, with adaptations of the apparatus developed by Wells (Fig. 2; see also Wells, 1949; Wells & Dales, 1951). These studies were made to determine how much time was occupied with feeding, and how feeding was related to other activities of the animal.

Irrigation of the burrow is almost incessant, provided that the openings of the burrow remain submerged. Both in glass tubes (Fig. 2), and in glass/mud/

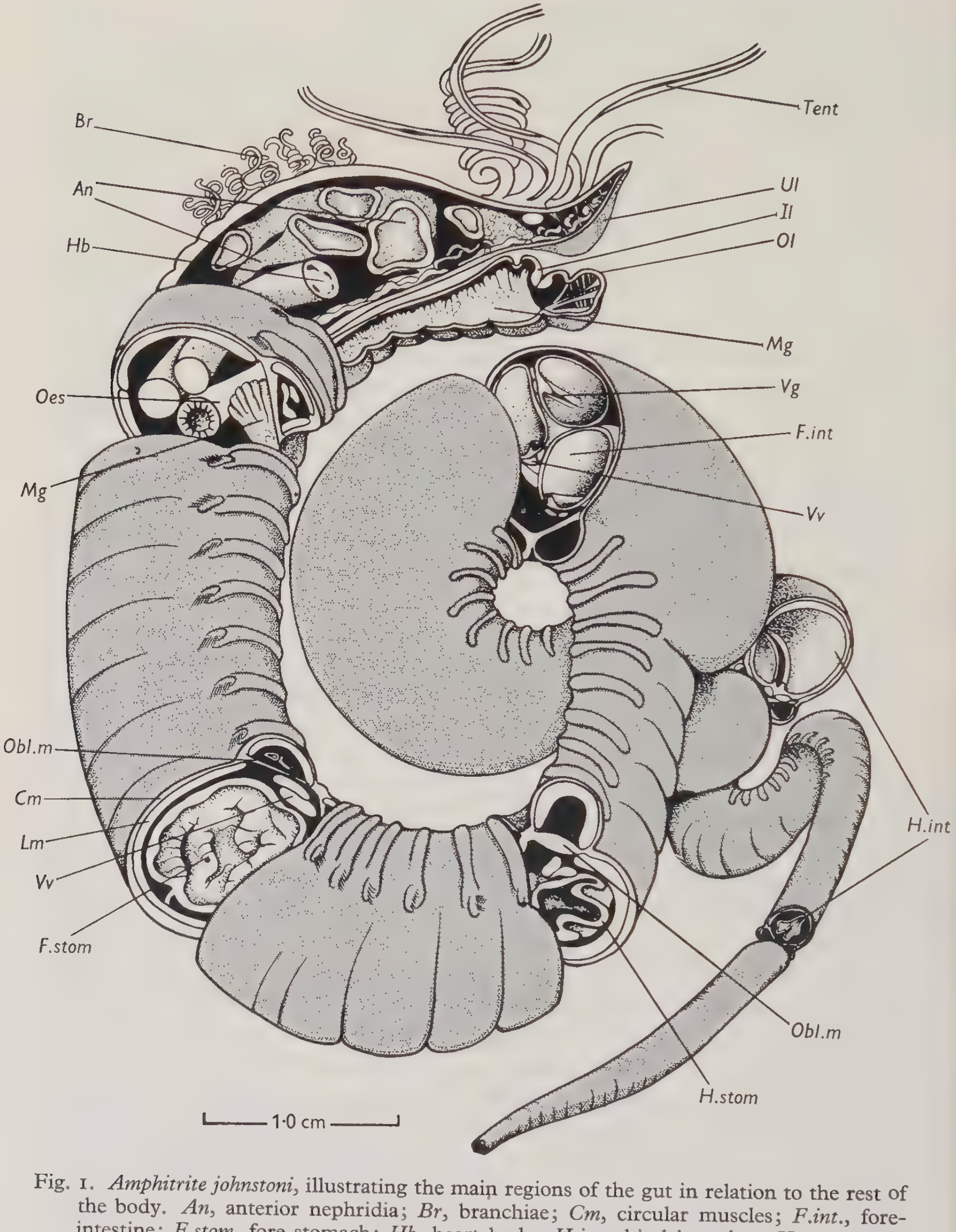
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Fig. 1. *Amphitrite johnstoni*, illustrating the main regions of the gut in relation to the rest of the body. *An*, anterior nephridia; *Br*, branchiae; *Cm*, circular muscles; *F.int.*, fore-intestine; *F.stom*, fore-stomach; *Hb*, heart body; *H.int.*, hind-intestine; *H.stom*, hind-stomach; *Il*, inner lips; *Lm*, longitudinal muscle; *Mg*, mucous glands; *Obl.m.*, oblique muscles; *Oes*, oesophagus; *Ol*, outer lips; *Tent*, tentacles; *Ul*, upper lip; *Vg*, ventral gutter; *Vv*, ventral vessels.

glass sandwiches the worm will irrigate the tube or burrow by peristaltic contractions of the body-wall initiated at the hinder end of the thorax and travelling forwards. At about 17° C each wave reaches, or almost reaches, the anterior end before the next wave is initiated, about 15 sec occurring between each wave. Such irrigation behaviour may continue for many hours with little other activity apart from slight stretching, or occasional forward extension of the body owing to a gradual slipping back during irrigation. Occasionally the direction of irrigation is reversed, the waves travelling back from the head, this usually occurring when active feeding is taking place. All the worms which were kept in the laboratory fed for about 50% of the time, weakly irrigating in a head to tail direction while doing so, and alternating such feeding periods with periods of much more vigorous and sustained irrigation from tail to head, during which the body is usually allowed to slip back in the tube until the hinder end of the body is almost exposed from the burrow opening. Defaecation usually occurs when the worm is in this position, the hinder end of the body being everted as much as 3–5 cm from the burrow opening; a faecal pellet is then ejected, and the body withdrawn immediately afterwards. This kind of behaviour was seen in all the terebellids studied. The type of record obtained with a worm lying in a horizontal tube is shown in Fig. 3.

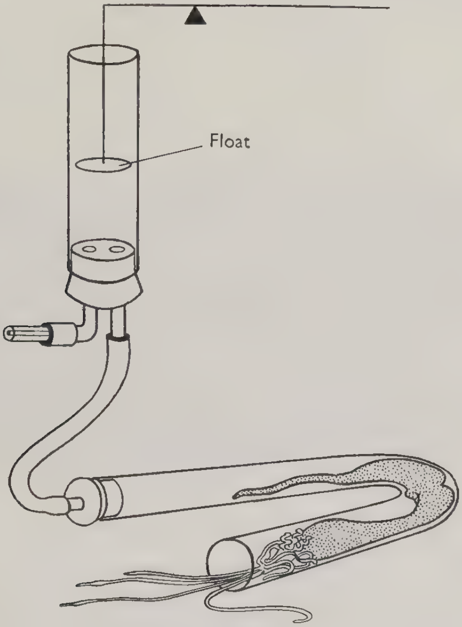

Fig. 2. Apparatus used for recording the behaviour of *Amphitrite johnstoni*. The whole apparatus is immersed in a tank, through which a flow of sea water is maintained at a constant level to that of the float.

The irrigation of the burrow can be made to cease immediately by connecting the two openings of the tube (see the apparatus used for *Nereis*, Wells & Dales, 1951). Such a constraint may resemble the conditions in nature when the burrows are uncovered by the tide. Actually this would happen only once or twice a month, but it is of interest to know how the worms react to such conditions. Such closure of the circulation for periods of 3–4 h always resulted in an almost complete cessation of irrigation activity, punctuated only by periodic bursts of 'testing' movements. These short bursts of irrigation waves last only a few minutes, usually in one, then in the other direction, followed by complete quiescence for some minutes or only by slight stretching movements. Worms never reversed themselves in the tube under these conditions. If a worm is placed in a U-tube only partly filled with water, irrigation activity is completely replaced by occasional exploratory movements. Immediately on opening the circulation or on immersion of the tube, vigorous irrigation results.

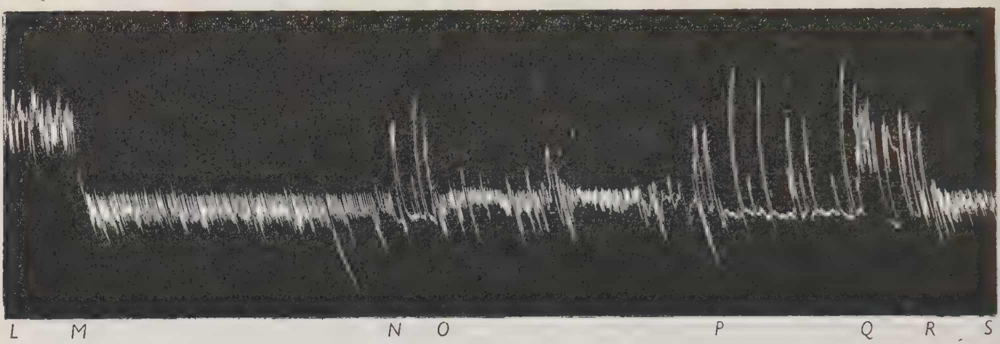

Fig. 3. Record of the behaviour of *Amphitrite johnstoni*, using the apparatus shown in Fig. 2. Duration of record, 11 h. Read from left to right. *L* to *M*, tail to head irrigation, head towards open end, at *M* worm turns round and irrigates tail to head, turning again at *N*; feeding *N* to *O* with gentle, steady head to tail irrigation, at *O* turns tail to opening and irrigates tail to head with some excursions up tube, turning again at *P* to feed; big spikes are due to excursions at mouth of tube during feeding, otherwise trace shows constant head to tail irrigation; at *Q* feeding ends and tail to head irrigation resumed (head still towards opening), turning at *R*. The frequent reversals are due to one end of the tube being connected to the recording apparatus.

In nature, *Amphitrite johnstoni* is commonly accompanied by a commensal scale-worm, *Gattyana cirrosa* (Pallas). The *Gattyana* is usually able to run forward in the burrow to rob the *Amphitrite* of part of its food since irrigation movements are reduced when ingestion occurs. When the terebellid is lying in the tube and irrigating, the *Gattyana* is usually quiescent, and commonly lies against the body of the terebellid just behind the thorax. Presence or absence of the *Gattyana* makes no discernible difference to the behaviour of the *Amphitrite*.

Feeding

Operation of the Tentacles

The tentacles are extremely extensile hollow structures with a complicated musculature enabling them to be moved in a great variety of ways. The adoral surface, comprising rather less than one-half of the total area, is ciliated, and this part usually forms a ciliated groove. All the cilia beat towards the mouth. The interor of the tentacle communicates with the anterior coelom, and movement of fluid within the cavity can easily be followed by observation of the coelomic corpuscles. Fig. 4 gives some idea of the structure and operation of the terebellid tentacle. While these illustrations refer to *Terebella lapidaria* L., the structure of the tentacle is in general features the same in all terebellids. Fig. 4 C is a stereogrammatic cross-section to show the main features of the musculature; Fig. 4 D illustrates some of the histological details.

The thick epidermal layer contains mucous cells, not only in the ciliated part, but throughout the epidermis. Longitudinal muscles extend the length of the tentacle but are not present where most lateral movements occur, that is, along the edges of the groove (*Lm*). Vertical or transverse muscles (*Tm*) link the middle of the ciliated area with the opposite side of the tentacle. In addition, there are oblique muscles (*Obl. m*) running in the direction of the axis of the tentacle, with their proximal insertions always on the side remote from the ciliated groove, and also individual muscle fibres (*Tmf*) spanning the inner and outer edges of the groove. The main longitudinal muscles, which enclose five longitudinal nerve strands (*Tn*) the median of which is the largest, are clearly for the retraction of the whole tentacle. The transverse and oblique muscles are responsible for the curling and rolling movements of the tentacle, while the individual fibres towards the edges of the groove cause the rippling and squeezing movements of the edges.

The great extensibility of the tentacles has been the subject of much comment. The comparison has been made between the terebellids with their highly extensile tentacles, and the ampharetids which possess rather similar tentacles which are not, apparently, very extensible. One of the differences between the two families to which attention has been directed in this connexion, is the presence in the terebellids of four conical pouches on the first diaphragm—this diaphragm cutting off an anterior coelom from the general body cavity. It was first suggested by Meyer (1887) that it is the contraction of these sacs and the forcing of fluid into the tentacles that causes their extension. The sacs are not principally muscular, however, and it seems unlikely that pressure of the fluid is itself responsible for the extension of the tentacles. By the dilation and relaxation of these sacs it may be that during the continual movements of the tentacles and the anterior part of the body, the coelomic pressure is maintained at a constant level ensuring continued normal working of the muscles in every position of the body. If a living worm is pinned down in a dish, and the anterior coelom opened, the worm will, after

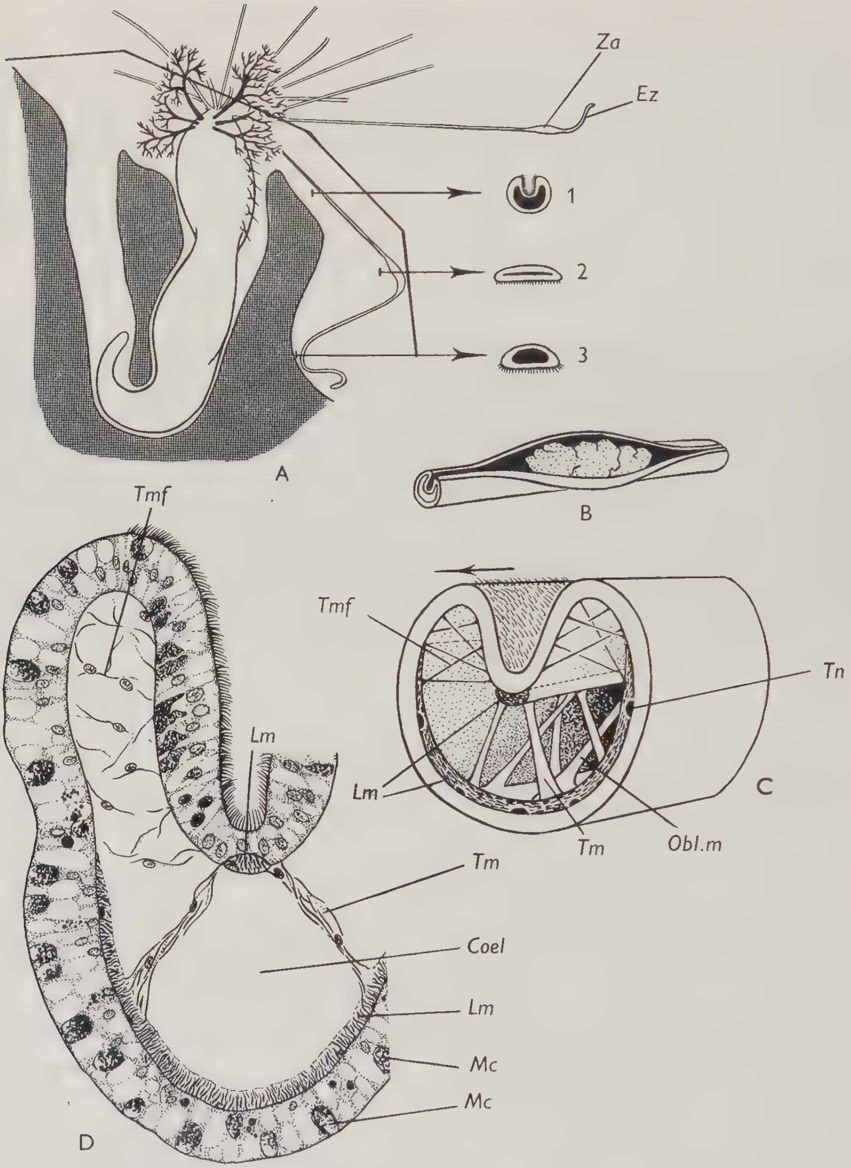

Fig. 4. Operation and structure of the tentacles in *Terebella lapidaria*. A, animal in feeding posture within its tube in the silt beneath a rock slab; *Za*, zone of attachment, *Ez*, exploratory zone; 1, appearance in cross-section in extended part of tentacle, 2, at zone of attachment, 3, in exploratory part. B, conveyance of particles along groove by ciliary action and squeezing by the transverse muscle fibres. C, stereogram of a tentacle to show muscle system, D, transverse section of tentacle to show histological details. *Coel*, coelomic space; *Lm*, longitudinal muscle; *Mc*, mucous cells; *Obl.m*, oblique muscles; *Tm*, transverse muscles; *Tmf*, transverse muscle fibres; *Tn*, tentacular nerves.

the initial shock period has passed, extend its tentacles almost as much as before, even though no pressure can be exerted by the coelomic fluid.

Most of the extension of the tentacle is due to ciliary creeping; by rolling over and opening out the ciliary groove in order to present a flat ciliated surface to the substratum, the tentacle can crawl along rather like a planarian. After a certain amount of extension the tentacle attaches itself to the substratum just behind the tip, by mucus, and perhaps also by suction caused by contraction of some of the transverse muscles. The tentacle is always flattened at this point (Fig. 4A, *Za*) and the cavity must be virtually occluded. This 'segmentation' of the tentacular cavity may be an important factor enabling the further extension of the tentacle, which is continually releasing and reattaching to the substratum a short distance behind the tip. In this way the tentacle follows a somewhat circuitous path thereby efficiently exploring the surroundings for food or debris. By the continual release and reformation of the 'point d'appui' the tentacle is being straightened and tautened, enabling the tentacle to be extended each time to its maximum extent. Small particles may be conveyed back along the ciliary groove when the tentacle is in this position, but sooner or later the tentacle is pulled in and re-extended in a new direction. In this way a large area can be gleaned of detritus.

In *Amphitrite johnstoni*, and in all the other terebellids that have been studied, the tentacles have been found to be insensitive to light. This is not so in all terebellids, as Welsh (1934) found in *Terebella gigantea* that the distal third of the tentacle was sensitive to light. All terebellids, on the other hand, are very sensitive to tactile stimuli, and the touching of one tentacle may cause a prompt retraction of all the tentacles.

Lip-mechanism

No particle can be conveyed direct to the mouth as the cilia do not extend to the base of the tentacle, and all particles are received first by the lips.

All particles collected by the tentacles are conveyed to the lips by one or more of the following methods.

(1) Very small particles such as diatoms, single sand grains and so forth may be conveyed along the groove of the tentacle by the action of the cilia. The groove may be partly open or completely closed so as to form a tube (Welsh, 1934, has also observed this in *T. gigantea*).

(2) Larger particles are usually conveyed along the groove partly by action of the cilia and partly by a squeezing action of the sides of the groove (Fig. 4B).

(3) Particles too large to be transported by either of these methods are usually secured by the sides of the groove and the tentacle then pulled in.

As already mentioned, the upper lip is formed from the prostomium. It is provided with muscle fibres running in different directions from the ventral to the dorsal side giving the lip a variety of movements (Fig. 5C–E). The oral surface is completely ciliated, and slightly grooved, the grooves running down towards the mouth. Beneath the mouth are two pairs of lips, the outer pair of which may be further subdivided so that a series of ridges is formed. The inner pair of lips is formed from the stomodaeum; the outer pair of lips is derived from the ventral part of the first segment. The two pairs of lips usually differ in appearance; the outer pair is ciliated and has mucous gland-cells in the epithelium, and in most terebellids is consequently yellowish or whitish in appearance. The inner lip-pair is usually reddish, and often grooved; these lips are never ciliated nor are there any mucous gland-cells in the epithelium (Fig. 5A, B). This series of lips can be recognized in all

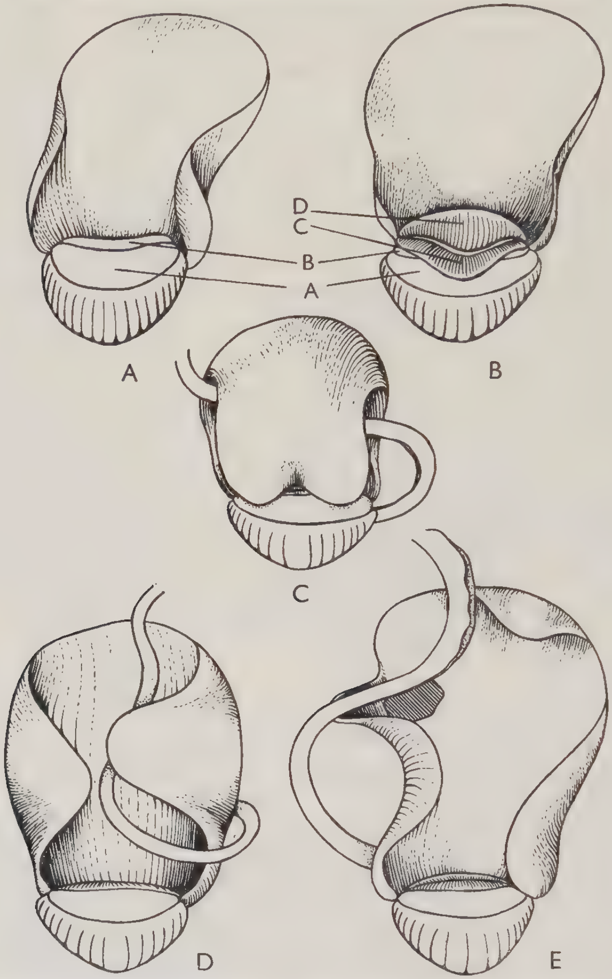

Fig. 5. Lip movement in *Amphitrite gracilis*. A and B show the rolling action of the inner lips; C–E, typical movements of the upper lip. A, B, C and D lips (see text).

terebellids, though they are variably developed. For convenience, the outer fold of the outer lip-pair is referred to here as 'lip A', the inner fold 'lip B'; the outer fold of the inner lip-pair, 'lip C', and the corresponding inner fold as 'lip D'. In some terebellids only the inner part of lip A functions as a lip, in the sense that it is directly concerned with the food, the outer part often being developed into a platform or flange concerned with tube-construction. A fifth lip fold, 'lip E', can usually be recognized inside lip D.

Some idea of the musculature of the lower lips can be had from Figs. 6 and 7C. The musculature of the outer lip-pair (A and B) is derived from the circular and longitudinal segmental muscles of the ventral side. Those on the dorsal side are not recognizable. The transverse or circular muscle coat is not well developed, and in *Amphitrite johnstoni* buckling movements of the outer lips in the transverse plane are not important in feeding. The longitudinal muscles, on the other hand, are very important, and these are attached in two main transverse fascicles externally visible as lips A and B, and these muscles may be further divided each into two groups so that a groove can be formed in each lip. It is the serial contraction of these longitudinal muscles that gives the outer lip-pair their characteristic rippling action. The lips forming the inner pair (C and D), on the contrary, are provided each with a large band of transverse muscle backed on the inside by a thinner band of longitudinal muscle running from the outer edge of lip C to the inner edge of lip D. The contraction of the longitudinal muscle makes these inner lips pout outwards, and when this is accompanied by a relaxation of the transverse bands, and is combined with the rippling action of the outer lip-pair, the inner pair of lips make a scooping movement into the hollow temporarily formed by the outer pair at the moment when lip B is somewhat contracted. This is illustrated in the diagram (Fig. 6). Here (Fig. 6A) both lip A and lip B are shown divided by the longitudinal muscles; though the lower part of lip A forms a parapet to the lip and is not really concerned with feeding. The circular or transverse muscles of the outer lip-pair are not shown here. The food-bolus arrives in the groove between A and B, the groove being deepened by contraction of the

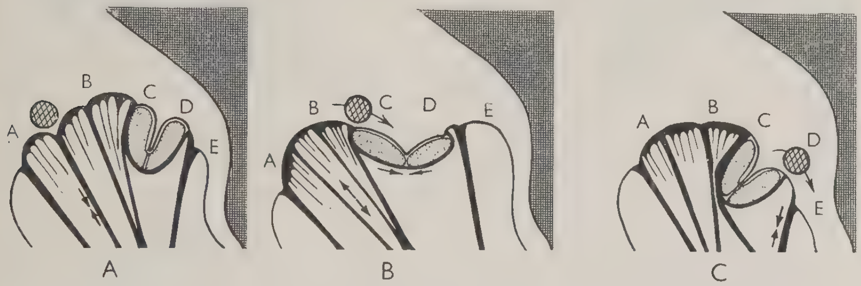

Fig. 6. Diagram illustrating the lip movements in *Amphitrite johnstoni*. For explanation see text.

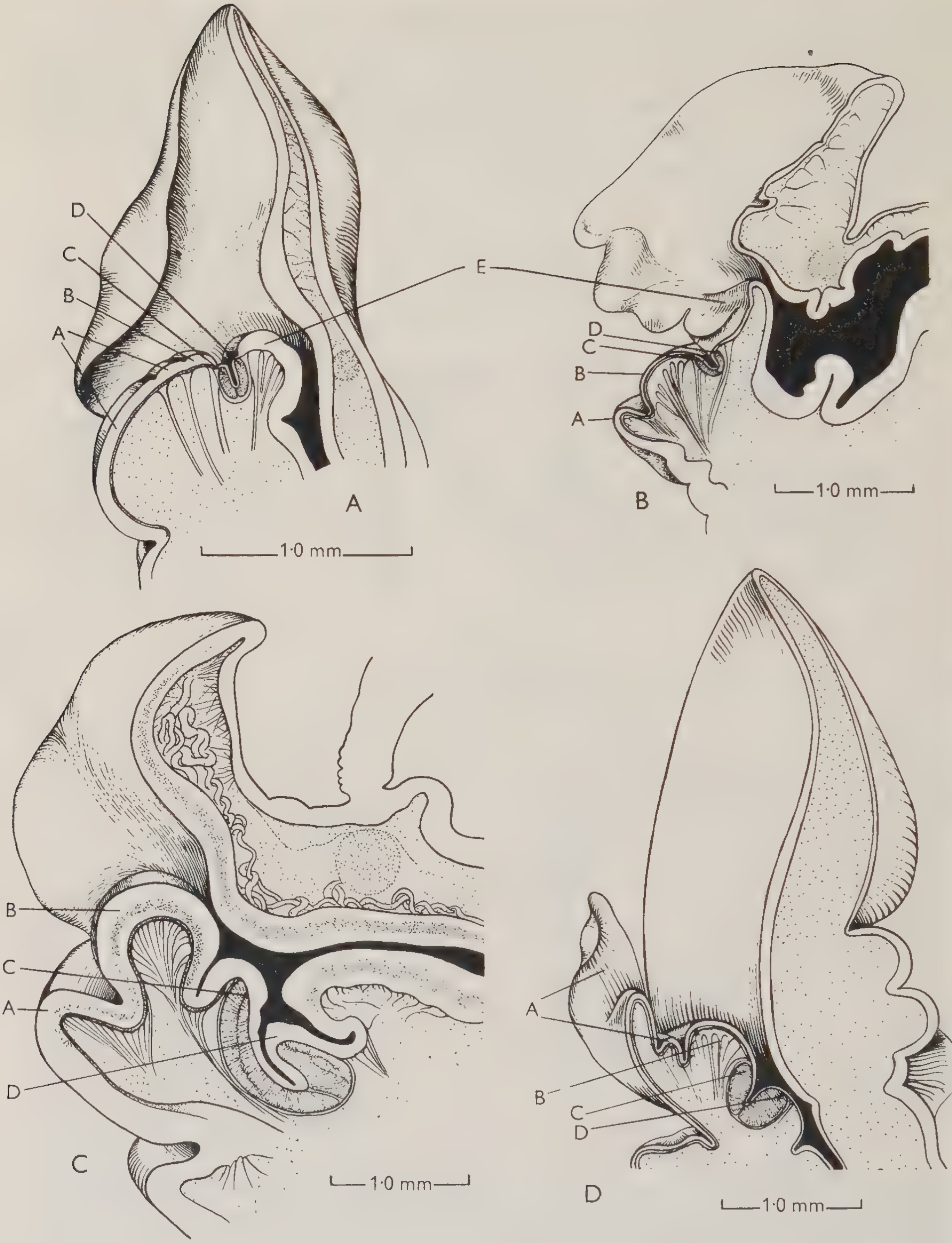
D
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Fig. 7. Comparison of lip structure in four terebellids: A, *Polycirrus aurantiacus*; B, *Terebellides stroemi*; C, *Amphitrite johnstoni*; D, *Lanice conchilega*. A, B, C, D and E, lips.

longitudinal muscles as shown. By a serial contraction of these muscles (from left to right in the diagram) the bolus is rolled forwards on to the outer rim of lip C (Fig. 6B). The inner lips receive it, opening out by contraction of the longitudinal muscle, close on to it, and roll forwards to the opening of the mouth by contraction of the longitudinal muscle between D and E, and also by some action of the transverse muscles of C and D which run round the sides of the mouth (Fig. 6C). This cannot be adequately shown in the diagram, and this explanation is much simplified. In practice the food may be in the form of a string running the length of the lip, or a rounded particle to one side. In consequence there is a great variety of curling movements to convey the food to the mouth.

It has been mentioned already that the outer lips, A and B, are ciliated. These cilia beat towards each other so that small particles alighting on either lip are swept down into the main groove between the two. In *A. johnstoni*, though not generally in other terebellids that have been studied, the tentacles tend to be wiped across these outer lips so that all the food particles are collected there. When a certain quantity has been collected, the inner lips scoop up the contents in the way already described. There seems to be little selection of the particles to be ingested. Obnoxious substances reaching the lips cause the complete retraction of all the lip surfaces. When these are again extruded, the unrolling action of the outer lips effectively pushes away the offending matter on to the ventral side of the body. Apart from this, virtually any particle of small enough size will be ingested, while particles which are too large cannot be scooped up by the inner lip-pair and are merely pushed off the outer lip groove. In most terebellids these are the particles which, having become covered with mucus from the lips A and B, are incorporated into the substance of the tube.

In practice the feeding movements of *A. johnstoni* do not always correspond exactly to the idealized behaviour described above, and in none of the terebellids studied are these movements very precise. Some of the particles arriving in the neighbourhood of the mouth may be wiped directly on to the inner lips, some on to the upper lip. Some of the movements regularly performed by the upper lip in *A. gracilis* are shown in Fig. 5. Small particles arriving on the upper lip will be conveyed directly down into the mouth by ciliary means. Larger particles will find their way down on to the lower lips, where they will be rolled either into the mouth, or off on to the outer lip parapet according to their size.

Morphology and Histology of the Gut

The mouth leads into a long narrow oesophagus which opens into a wide thin-walled fore-stomach. A pharyngeal region cannot strictly be distinguished. The fore-stomach opens into a hind-stomach, easily recognized by the thick layer of muscle giving it a glistening appearance, and passes to an intestinal region which leads to the anus. The anterior part of the intestinal region is coiled, and lies in the swollen part of the body immediately posterior to the thorax (Fig. 1, *F.int.*); the hind intestine is straight and supported by septa. All the septa in the thoracic region, and also in the part of the body occupied by the fore-intestine, have been reduced save for the single anterior septum mentioned previously. It is the reduction of the septa in this region that enables the fore-intestine to become coiled, the length of this part of the gut thereby being increased to 4 times the length of the part of the body in which it is contained. Although the septa are lacking in this region, the intestine and stomach are suspended by muscles attached to the mid-dorsal line of the body-wall (Fig. 8 C, *Dsm*). The muscles are well developed and may cause the slight, but possibly important, writhing movements of the intestine.

Internally the oesophagus is ciliated, and the epithelium contains many mucous cells closely resembling those described and illustrated by Brasil (1904) in *Pectinaria* (Fig. 8 D). Mucous cells are present in varying proportions throughout the gut with the exception of the muscular hind-stomach, but mucous cells are most numerous in the oesophagus and towards the hinder end of the posterior intestine which again is entirely ciliated. The epithelium of the oesophagus is thrown into folds. Near the mouth these form transverse ridges at right-angles to the axis of the oesophagus; farther back they form sinuous ridges parallel to the axis of the gut. The oesophagus has a well-developed layer of inner circular muscle and outer longitudinal muscle.

The fore-stomach is much less muscular, and the external layer of longitudinal muscle in this region and in the hind-stomach and fore-intestine is poorly developed but for one large strip running along the mid-dorsal line and related to the dorsal suspensory muscles. The circular muscles are also reduced in the fore-stomach but are, nevertheless, capable of causing constricting movements. Externally, as well as internally, the wall of this part of the gut is thrown into folds, and presents a yellowish appearance. Internally there are no ciliated cells, but the epithelium contains secretory cells of various kinds, and replacement cells towards the base. All the cells in this region have a brush-border (Fig. 8 E). Apart from the mucous cells most of the secretory cells present a vacuolated appearance the cytoplasm between the vacuoles being very granular (Fig. 8 E, *Sc* 1). There are also a few cells containing very large granules which stain red with azan and blue-black with iron haematoxylin, and presumably also of a secretory nature (Fig. 8 G, H, *Sc.* 2).

The hind-stomach contrasts strongly with neighbouring regions by virtue of the well-developed muscles in the gut wall. This muscle all belongs to the circular muscle layer, and in spite of contrary assertions in the literature, all the fibres run in a circular direction and may be separated into thin washer-like lamellae. The longitudinal muscle layer is mainly represented by the mid-dorsal strip already mentioned, but this is well developed and capable of strong contractions. Internally the epithelium is uniform, and consists of

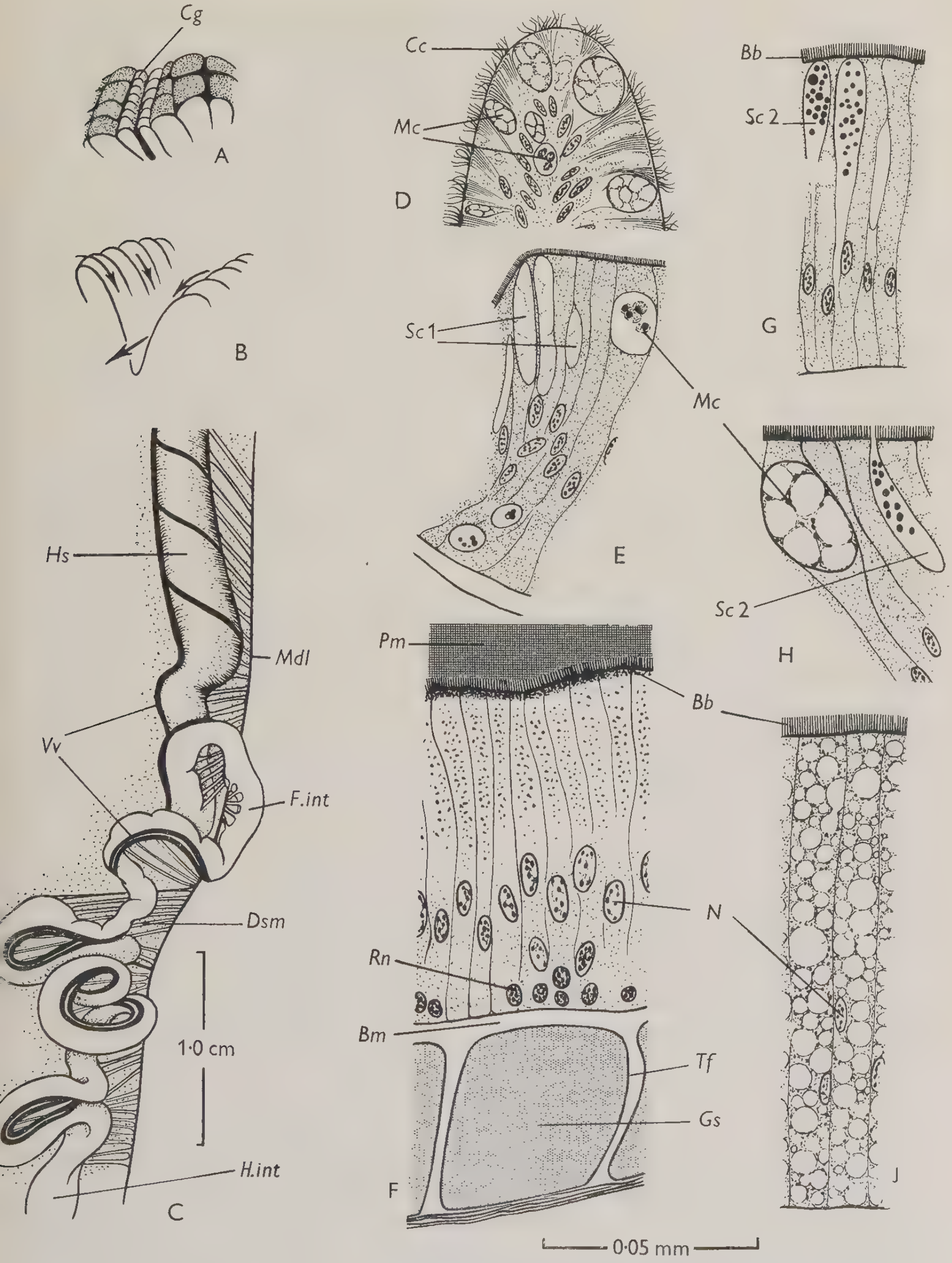
Cg
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Fig. 8. A, appearance of ciliary groove in life; B, ciliary currents within the groove; C, hind-stomach and fore-intestine in *Amphitrite johnstoni* to show coiling and dorsal suspensory muscles; D–H histology of the gut in *A. johnstoni*: D, oesophagus; E, fore-stomach; F, hind-stomach; G, H, fore-intestine; J, fore-stomach in *Polycirrus*. *Bb*, brush-border; *Bm*, basement membrane; *Cc*, ciliated cell; *Cg*, ciliary gutter; *Dsm*, dorsal suspensory muscles; *F.int*, fore-intestine; *Gs*, gut sinus; *Hs*, hind-stomach; *Mc*, mucous cell; *Mdl*, mid-dorsal line; *N*, nucleus; *H.int*, hind intestine; *Pm*, peritrophic membrane; *Rn*, nucleus of replacement cell; *Sc* 1, *Sc* 2, 1st and 2nd types of secretory cell; *Tf*, transverse fibre; *Vv*, ventral vessels.

cells with a very granular cytoplasm including larger heavily staining granules, and with a brush border (Fig. 8 F). The epithelium itself is separated from the contents of the gut by a thick peritrophic membrane presumably secreted by the epithelium. The membrane was shown to be composed of chitin by the chitosan-iodine test of van Wisserlingh and Campbell (Richards, 1951). As noted previously by Thomas (1940), the membrane is separated from the epithelium by a very thin layer of some other substance, presumably corresponding to what Newell & Baxter (1936) refer to as 'coagulum' in the ampharetid, *Melinna*. While ciliated and brush-borders have been carefully distinguished in the present work, special attention has not been given to the cytological details of the brush-border in different parts of the gut. However, it would be of interest to re-investigate the structure of the cell border in invertebrates such as terebellids, using the special techniques developed by Baker (1942) for his study of vertebrate intestinal epithelia.

The intestine is sharply distinguished from the hind-stomach, not only by the greatly reduced circular muscle coat, but also by the initiation of the median ventral ciliary gutter, which increases in size posteriorly. The ciliary gutter is the only part of the fore-intestine which is ciliated, all the other epithelial cells have a brush border. Throughout the intestine the left-hand ridge of the ciliary gutter, looking down the gut, is rather taller than that on the right. The cilia on the sides of the groove beat downwards towards the base, those along the base beat towards the anus (Fig. 1, *Vg*; Fig. 8 A, B). The anterior part of the intestine, as already mentioned, contains secretory cells, mainly of the type with large, heavily staining granules (Fig. 8 H, *Sc* 2); farther back there are fewer of these secretory cells and most of the epithelium is composed of cells with a fairly uniform granular cytoplasm. Mucous cells are scattered throughout.

The hind-intestine differs from the fore-intestine in the ciliation of the whole epithelium. Mucous cells are more numerous, and secretory cells of the type found in the fore-stomach and fore-intestine are absent.

As in other Polychaeta Sedentaria, there is a blood sinus enclosed within a connective tissue sheath between the epithelium and the circular muscle layer. The sinus is largest in the region of the hind stomach (Fig. 8 F) where strands of connective tissue cross the sinus.

Passage and Digestion of Food

By the time the food enters the mouth it is already wrapped into a mucous string or bolus, and is passed down the oesophagus by a combination of ciliary and peristaltic action. The circular muscle layer of the oesophagus is comparatively thick, and waves of peristalsis can be seen passing down the oesophagus in dissected living specimens.

Though food may be transported mainly by ciliary means in ciliary feeders such as serpulids and sabellids, the importance of the cilia in transporting food in the gut of other polychaetes has probably been overstressed.

Hanson (1948) has drawn attention to the direction of peristalsis in the gut of serpulids and sabellids. In small transparent individuals in which movements of the gut may be watched under the microscope, the direction of peristalsis is apparently always towards the mouth. This was reported earlier by Claparède (1873) and Stephenson (1913). In terebellids it is difficult to make direct observations on gut movements, but as the whole part of the stomach region is unciliated, transport of the contents in this region at least must be due to muscular action of some kind. Peristalsis in the oesophagus is clearly in a posterior direction; in the fore-intestine, only antiperistalsis has been seen, quite strong waves of contraction passing anteriorly, notwithstanding the relatively thin muscle layer in this region. These movements of the fore-intestine can be seen by transparency of the body-wall, and are quite distinct from the writhing movements of the whole intestine. In the fore- and hind-stomachs, strong constricting and segmenting movements may be seen on opening a worm, but these movements soon cease, and no definite peristalsis was ever seen in either region. However, the movements of the body-wall, and of the dorsal suspensory muscles, may have an important effect on the transport of the gut contents. The change in direction of the irrigation contractions of the body-wall from anterior to posterior during feeding, may be significant in this connexion. Certainly defaecation is accompanied by violent contractions of the hinder part of the body-wall, which presumably exerts a squeezing action on the hind-intestine. The slight movements of the body in the region of the fore-intestine which may be seen in a normal worm, are related to the writhing movements of the gut itself, presumably through the intermediacy of the dorsal suspensory muscles, and these movements may also be important in transport of the gut contents. Although Hanson (1948) may be right in assuming that the ventral ciliated groove is responsible for the transport of the gut contents posteriorly against the antiperistaltic action of the gut itself, the importance of the body-wall movements should not be underestimated. The ciliary gutter may be of use in rapidly removing indigestible material from the site of absorption. The excised hind-stomach, when suspended under slight tension in a bath of sea water, shows periodical longitudinal contractions which may be recorded kymographically. These contractions are probably mainly caused by the dorsal longitudinal muscle strip, and occurred at intervals of about 5 min in a fresh preparation. These movements may help in pumping the food from the fore-stomach to the intestine. The irrigation movements of the body-wall seem to be spontaneous, as Wells has found in many other polychaetes, a body-wall strip attached to the nerve cord showing very regular bursts of activity. No clear connexion between this activity and the gut movements could be detected in preparations in which a strip of body-wall and the different parts of the gut were linked to levers recording traces on the same drum. Transport of the gut contents is probably mainly muscular, and due to a variety of movements of the gut itself, and to the body-wall.

The pH of the oesophageal contents is distinctly lower than that of the outside medium, and it may be that some secretion of enzymes takes place towards the posterior end of the oesophagus, as a few secretory cells similar to those in the stomach are found there. The pH of the oesophageal contents is usually about 7·0; that of the fore-stomach is about 6·0 in a feeding worm, and this distinctly acid medium is maintained throughout the following regions of the gut, only rising significantly on entering the hind-intestine, where the pH rises gradually from 6·2 at the beginning of the septate region to about 7·2 at the rectal end. In starved worms the pH throughout the gut is much higher, and little different from that of the coelomic fluid (7·2–7·4).

Simple spot-tests for enzymes in the gut contents showed that a lipase was present in the fore-stomach and fore-intestine; strong amylase activity and rather weak protease activity in the fore-stomach, and rather stronger protease activity with some amylase activity in the fore-intestine. Tests on extracts of the washed tissues of the fore-stomach and fore-intestine taken from starving worms confirmed the impression that protease activity was stronger in the fore-intestine, and amylase activity stronger in the fore-stomach. Brasil (1904) found amylase activity only in the most anterior part of the mid-gut of *Pectinaria koreni* (corresponding to the fore-stomach in terebellids) and associated this with the 'claviform glands' he described (similar to Fig. 8E, *Sc* 1). Protease activity was found in the second part of the mid-gut (corresponding with the fore-intestine of terebellids), and this Brasil associated with the 'cellules à ferment' (similar to Fig. 8G, H, *Sc* 2) which are most numerous in this region. Lipase activity seemed to be weak in *Pectinaria*. He found also that the pH throughout the gut was slightly alkaline, but this may easily have been due to admixture with the strongly buffered body fluid, for, as Nicol (1931) points out, sampling the contents of the gut without admixture of the body fluid is difficult, and would be particularly so in a worm as small as *Pectinaria*. Brasil's tests were all done on extracts of the tissues. His findings are confirmed by these observations on terebellids, as the distribution of the two main kinds of secretory cell is much the same as he describes, and also corresponds with the site of amylase and protease activity. This distribution of secretory cells is similar in all the other terebellids studied; Fig. 8J shows the appearance of the fore-stomach of *Polycirrus aurantiacus* Grube in which all the cells are of the vacuolated kind. Digestion is probably entirely extracellular, as the epithelial cells throughout the stomach and fore-intestine have a brush-border, and no amoebocytes were found in the gut.

Absorption, as shown by uptake of iron from iron saccharate ingested by normally feeding worms, takes place also in the fore-intestine and in the anterior part of the hind-intestine. None of the iron was ever found in the cells of the fore-stomach. This was confirmed with *Terebella lapidaria* which was found to feed more readily on iron saccharate.

EVOLUTION OF FEEDING MECHANISMS IN TEREBELLIDS

Feeding and Lip-Structure

All terebellids have the basic lip-structure which has been described, consisting of an inner and outer pair of lips below the mouth, and a single lip above. While the two pairs of lower lips may be recognized in all the genera which have been studied, the relative proportions and the development of each of these lips vary considerably, and these variations are related to different habits of feeding and of tube-building.

There is no doubt that the tentacles and the upper lip are derived from the prostomium, and not from the first segment as stated by McIntosh (1922). This is clear from the studies of Salensky (1883) and Wilson (1928) on the larval development, and from Nilsson's (1912) study of the nervous system. There is also no disagreement among morphologists that the first segment (peristomial segment, 'segment buccal'), while chaetigerous and possessing a nephridium in the larva (Wilson, 1928), is greatly reduced in the adult, where all that remains are the parts forming the outer lips A and B, and the ventral musculature. In some forms, the lateral lips or collar which extend round the dorsal side of the body and which are usually adaptations for tube-building, are also derived from the first segment.

The inner lips (C and D), which have been referred to as the 'Schlundsac' by Meyer (1887) and the 'buccal organ' by Elrington (1909) and Wilson (1928), correspond to the 'muscle bulb' described in various archiannelids by Jägersten (1947). This organ is a very distinct structure and is usually red in colour in adult animals. It is outside the scope of the present paper to discuss the relations of this structure in the various families of Polychaeta Sedentaria, and its relationship with the eversible proboscis of the Errantia, but it may be noted that the structure is found throughout the Sedentaria occurring in families whose members have feeding habits very different from those of terebellids.

Four examples to illustrate the variation in lip structure are shown in Fig. 7. Of these, *Amphitrite johnstoni* (Fig. 7C) and *Terebellides stroemi* Sars (Fig. 7B) are found in mud or muddy sand; *Polycirrus aurantiacus* (Fig. 7A) is found under stones and in rock crevices, and *Lanice conchilega* (Fig. 7D) occurs in sand. Of these, *Lanice* is the best tube-builder, *Terebellides* does not really build tubes, although particles rejected in feeding and those in contact with the body become agglutinated to form a sleeve in which the worm lies. Neither *Polycirrus* nor *Amphitrite johnstoni* lives in tubes, the latter living in an unlined burrow in mud as described above. *Polycirrus* is more mobile and pulls itself about under stones by the tentacles, which are extremely numerous and completely hide the whole of the anterior part of the body; it is only by the amputation of the majority of the tentacles that feeding may be observed in this species. *Terebellides*, on the other hand, has extremely short tentacles, which are used much less for feeding than in most terebellids.

In *Lanice*, the upper lip is chiefly employed for building the tube—the lip is extremely flexible, and as described by Arnold Watson (1890) is the organ which is mainly responsible for laying in place the grains of sand and shell which constitute the tube, after having been plastered with mucus by the lower lips. The lower lip A is also expanded into a kind of collar, and this is used in applying more mucus to the inside of the tube as the rim is extended or repaired. As will be seen, it is the outer part of lip A which is thus modified, and rolling movements can still be executed during feeding by the inner part of A, by B, and the inner lip-pair, as in *Amphitrite johnstoni*. In *Lanice*, particles which are small enough to be ingested find their way down into the 'gullet' formed between the upper and lower lips and are accepted by the inner pair of lips and swallowed. Large particles will come to rest between the upper lip and the outer part of lip A, and these will be either incorporated into the tube or rejected according to suitability or need. It will be seen that C and D are quite well developed, though perhaps not so well as in *Amphitrite johnstoni*. Contrast, on the other hand, the size of the upper lip in these two species, one of which does, the other which does not, build a tube.

Unlike *Amphitrite johnstoni* and *Lanice*, *Polycirrus*, living as it does under rocks, is usually in the prone position while feeding. The very large upper lip is commonly curled into a partly closed tube or scroll, through which the tentacles are drawn. Particles collected under the upper lip are periodically pushed into the mouth by the movements of the lower lips, of which the outer pair A and B are the better developed, A forming a long mucus-producing tongue. The much greater use of the upper lip and of lip A, as compared with *Amphitrite johnstoni*, for example, is clearly reflected in the proportions of the parts concerned. Particles which may be gathered by the tentacles but which are too large to be ingested are simply pushed away by the rolling movements of A and B.

In *Terebellides* the lip structure has been profoundly modified (Fig. 7B). The upper lip is extended into a complete funnel joining laterally with the lip E. Both pairs of lower lips are almost excluded from the mouth, but the funnel in *T. stroemi* is much lower in the mid-ventral line, and the rolling movements of lips A–C may still help to push food collected in the funnel into the mouth. In the Antarctic species *T. minutus* the funnel is even more complete. As mentioned already, the tentacles are short in this genus, and *Terebellides* has been described as scooping up the mud on which it feeds with the ventral side of the funnel. Unfortunately *Terebellides* is not found intertidally on our shores, and I have not had the opportunity of studying the feeding movements, or those of *Artacama*, another aberrant genus to which reference will be made later. Both genera are cold water forms found in the Arctic and Antarctic regions, but Thorson (1946) states that they are the commonest bottom polychaetes in the Øresund. Hessle (1925), working in Sweden, was apparently familiar with *Terebellides*, and he writes: '*Terebellides*...gräbt mit der grossen zweilobierten Platte unterhalb des Mundes'. The 'zweilobierten Platte' clearly refers (from his fig. 3) to the ventral parts of the funnel, which in his figure is shown buckled up into a kind of scoop. If this movement is accompanied by the usual rolling of the lower lips, mud may be pushed into the mouth. Later, in the same context, he writes: 'Bei *Terebellides* werden sie aber an dem zweilobierten Hautkamm unterhalb des Mundes aufgenommen. Da dieser Kamm ein wenig vom Munde entfernt ist, gelangen die Partikeln nach dem Kamm durch eine Rinne, die dadurch zu Stande kommt dass der vordere Teil des Mundbodens sich eine Tülle bildend ausstülpt. Von der Spitze dieser Tülle fallen die Partikeln auf den Kamm.'

Artacama seems to have taken digging even further. In this genus, lip A has been enormously expanded into a papillated 'proboscis' which is presumably used for digging (Fig. 9). Lip B is fairly well developed, but the inner lip pair (C and D) are relatively small, and this suggests that these inner lips are not as important as in *Amphitrite johnstoni*, for instance. Like *Terebellides*, *Artacama* is a mud-dweller, and while the tentacles are not as short as in the former genus, they are certainly not as long as in some other terebellids. Genera such as *Terebellides* and *Artacama* are relatively deep water forms, and it may be that these rather different feeding habits are correlated with the different kind of deposit from that occurring around low-water mark.

Terebellides, and its near relatives, does, in its lip structure, come close to the ampharetids, which though differing in other ways, also have a funnel-shaped upper lip excluding both pairs of lower lips (see Fauvel, 1897). Hessle (1917) postulated a separate family for these genera, which he called the Trichobranchidae, and from which he derived not only the terebellids, but also the amphictenids and ampharetids. In lip structure *Terebellides* is more primitive than Hessle's type-genus *Trichobranchus* which has the upper lip cleft ventrally to allow the lower lips to roll into the mouth. For many anatomical reasons it is unlikely that the terebellids arose directly from the ampharetids, and Hessle is probably correct in regarding *Terebellides* and its near relatives as closely approaching the stem form. The conclusion is therefore reached that the most advanced terebellids are those with highly developed tentacles and tube-building habits, and that the group has been derived from tubeless forms, living and feeding on mud.

Variations in Gut Morphology

Regarding the gut itself there is little variation in morphology or in the proportions of the parts, and no significant differences in histology. The oesophagus in all species is remarkable for its length. This is probably related at least in part to the very different morphology of the anterior region of the body; the large ventral mucous glands, the nephridia and heart-body take up much space in the coelom, and an expanded gut could not well be accommodated.

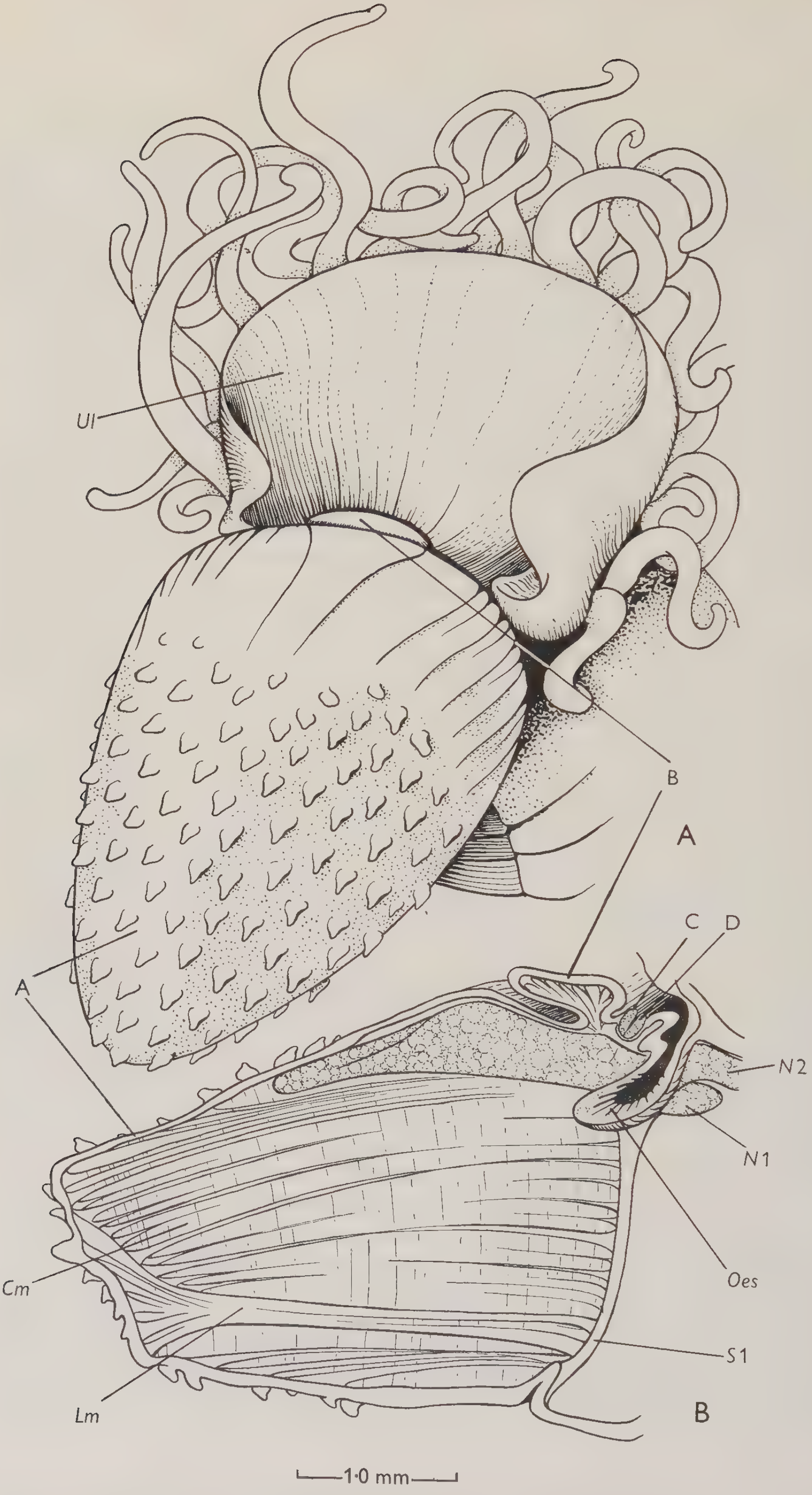
Ul
B
A
C
D
N2
N1
A
Oes
Cm
S1
Lm
B
1·0 mm

Fig. 9. *Artacama proboscidea*. A, external appearance of the 'proboscis' and lips; B, stereogram of a sagittal section through the proboscis. A, B, C and D, lips; *Cm*, circular muscle; *Lm*, longitudinal muscle; *Ul*, upper lip; *Oes*, oesophagus; *S*1, 1st septum; *N*1, *N*2, 1st and 2nd anterior nephridia.

Although the length of the muscular hind-stomach seems to be very variable, these differences are more apparent than real. Thus in all the species examined from the smallest, such as young *Polycirrus*, to the very large fully grown *Amphitrite edwardsi*, the fore-stomach represents 10–15% of the total length of the gut, the muscular hind-stomach 5–12%, and the intestine 65–75%. On the other hand, the epithelial area of the fore-stomach in *Terebellides* (and also of its near relative, *Octobranchus* (McIntosh, 1922)), is much increased by being thrown internally into folds and extended forwards as slight lateral lobes partly enveloping the oesophagus. In this, the gut resembles that of ampharetids, but there is no muscular stomach in the latter group (Fauvel, 1897), though there is a peritrophic membrane (Newell & Baxter, 1936). Wirén (1885) illustrates the gut of *Artacama* as being much coiled, with the oesophagus reflexed into the 'proboscis'. This may be an effect of fixation, as the animals which have been dissected during the present investigation did not have the oesophagus reflexed in this way.

There are no diverticula or oesophageal pouches in terebellids, as there are in many errant polychaetes. The 'salivary glands' mentioned by Quatrefages (1865) were probably nephridia, and the 'glandular organ entering the alimentary canal dorsally at the base of the oesophagus' in *Lanice* referred to by McIntosh (1922) is presumably the heart-body.

Bearing in mind the different feeding habits and the probable ancestry of the group it is interesting to note that larvae of terebellids have a simpler gut, the muscular hind-stomach being recognizable only when the young worm has adopted the adult mode of life (Salensky, 1883; Elrington, 1909; Wilson, 1928; and Thorson, 1946).

SUMMARY

The feeding and the functional morphology of the gut of *Amphitrite johnstoni* are described, and later compared with that of other terebellids. Feeding is effected by the tentacles bringing particles to the lips, where some sorting occurs, and the control of movement of the tentacles and the lip mechanism are described. The gut in all terebellids consists of an oesophagus, fore-stomach (comprising 10–15% of the total gut-length), muscular hind-stomach (5–12%) and intestine (65–75%). The muscular hind-stomach acts as a mixer and contains a peritrophic membrane. Enzymes are secreted by the fore-stomach and the fore-intestine. Strong amylase activity, weak protease activity and lipase activity is found in the fore-stomach; strong protease activity, amylase and lipase activity is found in the fore-intestine, and these differences are correlated with differences in histology. The gut has a pH of 6·0 in the digestive regions in feeding worms. Absorption occurs in the fore-intestine and the anterior part of the hind-intestine. Transmission of food throughout the gut is caused mainly by muscular action. The lip structure of *Amphitrite*, *Polycirrus*, *Lanice*, *Terebellides* and *Artacama* is briefly described in relation to the different habits of these genera.

APPENDIX

Notes on the Methods Used

This study has been based on an examination of the following species in particular: *Amphitrite johnstoni* Malmgren, *A. edwardsi* (Quatrefages), *A. gracilis* (Grube), *Terebella lapidaria* (Kahler), *T. californica* Moore, *Lanice conchilega* (Pallas), *Loimia medusa* Sav., *Polymnia nesidensis* (Delle Chiaje), *Nicolea venustula* (Montagu), *Pista cristata* (Müller), *Thelepus cincinnatus* (Fabricius), *T. crispus* Johnson, *Polycirrus aurantiacus* Grube, *P. caliendrum* Clap., *Trichobranchus glacialis* Malmgren, *Terebellides stroemi* Sars, *T. minutus* Hessle, *Artacama proboscidea* Malmgren.

Material for sectioning was fixed mainly with Bouin's and Gilson's fluids, cut at 7·5 μ and stained with Heidenhain's azan, or iron haematoxylin with mucicarmine, alum carmine, or Orange-G. Mann's methyl-blue-eosin was also used. 10% neutral sea-water formalin was used for fixing tissues prior to testing for absorbed iron after feeding with saccharated iron carbonate as recommended by Glick (1949). These sections were counterstained with Orange-G (in absolute alcohol) as this stain forms a good contrast with the blue of absorbed iron.

The B.D.H. capillator method was used to measure the acidity of the gut contents, bromothymol-blue (pH 6·0–7·6) and bromocresol purple (pH 5·2–6·8) covering the pH range found. This method gives a value to the nearest 0·2 pH on very small quantities. When measurements were made, the different parts of the gut were ligatured, the whole gut dissected out, dried on filter-paper to avoid contamination with coelomic fluid, and the contents withdrawn from each region with a fine glass pipette.

The presence of proteases in the gut contents was demonstrated by the ability to digest gelatin in the form of pieces of photographic film; amylases by testing for remaining starch after incubation of the sample in starch solution, and lipases by action on olive oil stained with Nile blue.

Details of the apparatus used for 'closing the circulation' are to be found in Wells & Dales (1951), Fig. 1. (*Nereis*), and the arrangement of the glass mud/glass sandwich apparatus from Wells (1949), Fig. 2.

Drawings of histological and anatomical details have been prepared with the aid of a camera lucida.

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VITAMIN A AND CAROTENOIDS IN CERTAIN INVERTEBRATES

III. EUPHAUSIACEA

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(Text-figs. 1 and 2)

INTRODUCTION

Our published work has shown that the northern euphausiids, *Meganyctiphanes norvegica*, *Thysanoessa raschii* and *T. inermis*, contain much higher concentrations of vitamin A than we have found in any other marine Crustacea (Kon & Thompson, 1949*a*; Batham, Fisher, Henry, Kon & Thompson, 1951; Fisher, Kon & Thompson, 1952, 1953, 1954). In the antarctic species, *Euphausia superba*, the concentration of vitamin A in samples taken from the alimentary canals of baleen whales (Thompson, Ganguly & Kon, 1949; Kon & Thompson, 1949*b*) was similar to that found in *Meganyctiphanes norvegica* from the gut of arctic baleen whales (Fisher *et al.*, 1952), but both were very much lower than in free-swimming *M. norvegica*. No corresponding free-swimming specimens of *Euphausia superba* had been analysed.

This evidence indicated that the Euphausiacea, as a group, might be richer in vitamin A than other Crustacea. We have, therefore, attempted to obtain as many other euphausiid species as possible for a comparative study, and in fact have now information about eight further euphausiids. Unfortunately, we found no other environment as favourable as Loch Fyne or Monaco for catching easily large numbers of these animals. The numbers of specimens analysed of these species were, therefore, relatively small, but, in most instances, valid results were obtained.

We have now studied samples of *Meganyctiphanes norvegica* from several localities, and the analytical results will be compared to illustrate any geographical variations in the vitamin A and carotenoid concentrations in this species.

During our study of *M. norvegica* (Fisher *et al.*, 1953) we noted certain discrepancies in vitamin A concentrations from different groups, and these we attributed to possible impurities in the preservatives used. We discovered, however, similar differences in the vitamin A content of various groups of *Euphausia pacifica* (see p. 83) where the quality of the preservative was not in doubt and performed therefore some experiments on *Meganyctiphanes* *norvegica* and *Thysanoessa raschii* collected from Loch Fyne to elucidate the problem. The results are given in a separate section immediately following those of the systematic studies.

MATERIAL COLLECTED

In all, eleven species of euphausiids were collected, some by ourselves and others by marine biologists on various research cruises or whaling expeditions.

Meganyctiphanes norvegica (M. Sars) was the most widely obtained species. Apart from our own collections from Loch Fyne (Fisher *et al.*, 1952, 1954) and from Monaco (Fisher *et al.*, 1953), several groups of this species were collected for us by Dr J. H. Fraser during a cruise of the Scottish Home Department's Fisheries Research ship *Scotia* in August 1951. During his researches on whale-marking at the Norwegian whaling station at Steinshamn in July 1953, Mr Robert Clarke collected samples of this species from the stomach of a 55 ft. ♂ Fin Whale.

Thysanoessa raschii (M. Sars) is a fjord-dwelling species (Einarsson, 1945) and we have obtained specimens only from Loch Fyne.

T. inermis Kröyer lives in more open waters but, apart from the groups that showed an apparent relationship between vitamin A concentration and depth, taken near the Faeroes (Fisher *et al.*, 1952) we have analysed no other free-swimming specimens. A sample of this species was, however, taken from the throat of a 72 ft. ♂ Blue Whale by Mr Clarke at Steinshamn.

Several samples of the antarctic species, *Euphausia superba* Dana, have been analysed. Specimens were collected for us during the cruise of the R.R.S. *Discovery II* in 1950–51, others were taken by tow-net from the whaling factory ship *Balaena* during her 1951–52 expedition, by Mr S. Brown and Mr R. M. Brachi, and another group of specimens was collected during the 1952–53 expedition of this ship by Mr Brachi and Mr H. W. Symons. Some of this last group were collected from the engine-room inlets of the *Balaena* and others by net from a meat-carrying ship.

During a cruise of R.R.S. *Discovery II* in the North Atlantic in August and September 1952, Mr Foxton obtained for us specimens of *Stylocheiron elongatum* G. O. Sars and *Thysanopoda acutifrons* Holt & Tattersall.

While visiting the United States in 1953, one of us (S. K. K.) spent 2 weeks at the Scripps Institution of Oceanography at La Jolla, California, and took part in a 3-day cruise (25–27 May) of the research vessel *Horizon* in waters some 150 miles west from San Diego, during which five species of Pacific euphausiids were collected with a 1 m closing net at depths from 400 m to the surface. These were *Stylocheiron maximum* Hansen, *Euphausia pacifica* Hansen, *Nematoscelis difficilis* Hansen, *Thysanoessa gregaria* G. O. Sars and *T. spinifera* Holmes.

METHODS OF PRESERVATION

The euphausiids collected from Loch Fyne and Monaco were preserved by boiling in sea water, as previously described (Fisher *et al.*, 1952, 1953), and by keeping the specimens in deep-freeze during the journey to this laboratory. The Monaco specimens were brought, frozen, by air and so were only a few hours out of deep-freeze. Eyes and bodies were separated at Shinfield and the parts were placed in absolute alcohol and stored in the deep-freeze.

The Pacific specimens were preserved by boiling and storage in the ship's refrigerator until the return to La Jolla. With the exception of two of the three groups of *Euphausia pacifica* collected, eyes and bodies were then separated in the laboratory and preserved in absolute alcohol. The two groups of *E. pacifica* were preserved whole in absolute alcohol. All this material was kept in cold storage until despatched to Shinfield. It was at atmospheric temperature for the 6 weeks during which it was in transit.

The specimens collected by Mr Clarke at Steinshamn were preserved in absolute alcohol, and those of Dr Fraser on the *Scotia* by boiling and refrigeration. Both groups were sent to us by post. On arrival here, the alcohol specimens were immediately deep-frozen, and the boiled specimens, which appeared to be in good condition after 2 days in postal transit from Aberdeen, were dissected into eyes and bodies for separate preservation in alcohol in the deep-freeze. Specimens were sorted into size-groups.

Groups of *E. superba* collected from the *Balaena* were preserved by boiling and storage in the ship's deep-freeze. Those brought back in May 1952 were sent to us from Liverpool, where the ship docked, by post. The specimens collected in 1953 were kept in deep-freeze on arrival at Liverpool and were transferred in an insulated vehicle to similar storage in London whence they were collected and brought back to Shinfield in an insulated container. Both consignments were separated into eyes and bodies and the 1953 specimens were also size-grouped by length in the way previously described (Fisher *et al.*, 1952, 1954).

Specimens of *E. superba* collected from the *Discovery II* were separated into eyes and bodies immediately after catching and the parts were preserved in absolute alcohol. Unfortunately these specimens were inadvertently stored in a cupboard where the temperature was rather high. The euphausiids collected during the north Atlantic cruise of the *Discovery II* were preserved by boiling and storage in the refrigerator until arrival at Plymouth. They were sent to us by post and arrived in good condition. After dissection into eyes and bodies, which were preserved separately in absolute alcohol, the specimens were stored in the deep-freeze.

In this study we have found that the eyes of *E. pacifica*, when analysed separately from the bodies, had a high concentration of vitamin A, but no vitamin A at all was present in the animals preserved whole. We noted similar discrepancies in our work on *Meganyctiphanes norvegica* from the Mediterranean (Fisher *et al.*, 1953). To investigate further this loss of vitamin A from whole preserved animals, experiments were now done on *M. norvegica* and *Thysanoessa raschii* collected from Loch Fyne on 21–22 October 1953. The specimens were separated into groups of uniform size, 23–25 mm long for *Meganyctiphanes norvegica* and 11–13 mm long for *Thysanoessa raschii*. These groups were then divided into smaller lots, each of twenty-five specimens of *Meganyctiphanes norvegica* or of twenty specimens of *Thysanoessa raschii*. Of sixteen such lots for each species, eight were boiled and eight left raw. From each group of eight lots four were separated into eyes and bodies and four left whole. All the material was then preserved in absolute alcohol. Two lots of boiled whole animals and corresponding groups of bodies and eyes and one lot of raw whole animals and corresponding bodies and eyes were kept at room temperature and all the rest of the material was stored at −25° C in the deep-freeze. Analyses were done at intervals on successive groups.

Analytical Methods

The same method of analysis for carotenoids and vitamin A was used as reported previously (Fisher *et al.*, 1952). When the samples analysed were sufficient for the purpose, as with *Nematoscelis difficilis*, *Thysanoessa inermis*, *T. raschii*, *Meganyctiphanes norvegica*, *Euphausia pacifica* and some groups of *E. superba*, chromatography on alumina columns was done before saponification in order to separate vitamin A ester from vitamin A alcohol. With species of which we had only a few specimens, only one chromatography was done, after saponification, and vitamin A was estimated all in the alcohol form. The species treated in this way were *Stylocheiron elongatum*, *S. maximum*, *Thysanoessa gregaria*, *T. spinifera*, *Thysanopoda acutifrons*, and the remaining groups of *Euphausia superba*.

Results

Meganyctiphanes norvegica (*M. Sars*)

Specimens from Whales. Two samples of krill, identified as *M. norvegica*, from the stomach of a 55 ft. 5 in. ♂ Fin Whale, were analysed. The results are given in Table I. The concentrations of fat and vitamin A were considerably higher than in previous specimens of this species from whales (Fisher *et al.*, 1952), although the astaxanthin concentrations were similar to those in earlier analyses. The krill had probably been more recently swallowed by the whale than on the previous occasions and less of the vitamin A had been leached out of it.

Specimens from Different Localities. As previously mentioned, we have now information about specimens of *M. norvegica* from several localities. Taking into account the effects of size and season (Fisher *et al.*, 1952, 1954), it is now possible to compare some of the groups. Some of the results shown in Tables

II–IV have been previously reported (Fisher *et al.*, 1953, 1954). In Table II, groups of specimens collected in the Sandy Bank area (61° 54′ N., 05° 45′ W.), near the Faeroes, are compared with groups of similar weights collected in Loch Fyne in November 1950. Fat, vitamin A and astaxanthin were all in similar concentrations in specimens from both areas, apart from two Loch Fyne groups with rather higher concentrations of astaxanthin. In Table III are compared groups of animals collected during August 1951, at a station in the North Atlantic, in the port of Monaco and in Loch Fyne. Whereas the concentrations of astaxanthin were nearly the same in each set of size-groups from the different localities, the vitamin A reserves of North Atlantic specimens were lower than of those from Loch Fyne or Monaco. The fat concentration in the Monaco specimens was much lower than in those from more northerly waters. Table IV gives the analytical results for groups collected from Loch Fyne and the port of Monaco in January and February 1952. In January the concentrations of vitamin A and astaxanthin were higher and the fat concentrations lower in Mediterranean than in Loch Fyne specimens. In February the astaxanthin concentrations were again higher in the Monaco specimens but the difference between the vitamin A reserves in specimens from the two localities was reduced. The fat concentrations of these two sets of groups were similar.

TABLE I. OIL PER CENT, VITAMIN A PER GRAM AND PER GRAM OIL AND ASTAXANTHIN PER GRAM AND PER GRAM OIL IN EUPHAUSIIDS FROM WHALES AT STEINSHAMN, NORWAY

Species	Weight (g)	Oil (%)	Vitamin A i.u./g	Vitamin A i.u./g oil	Astaxanthin µg/g	Astaxanthin mg/g oil
Specimens from stomach of 55 ft. 5 in. ♂ Fin Whale, collected 17. vii. 53						
Meganyctiphanes norvegica	26	2·2	7·0	310	41	1·8
M. norvegica	22	2·4	9·8	405	62	2·6
Specimens from throat of 71 ft. 8 in. ♂ Blue Whale, collected 16. vii. 53						
Thysanoessa inermis	177	0·9	9·3	1121	32	3·9

No β-carotene was detected in either species.

Range of Vitamin A Concentrations. We have now analysed 258 groups of *M. norvegica*, selected by length at 2 mm intervals, in the way previously described (Fisher *et al.*, 1954). The number of specimens per group varied between 1 and 359 and the vitamin A concentrations between 3·1 and 260 i.u./g. The histogram in Fig. 1 shows the number of groups at each concentration and the bulk of these lies between 10 and 20 i.u./g, the average for the whole series, weighted for the numbers of specimens in the groups, being 15 i.u./g. The very high concentrations, namely, those over 100 i.u./g, occurred in only a few groups, all of larvae (see Fisher *et al.*, 1954).

TABLE II. OIL PER CENT AND VITAMIN A AND ASTAXANTHIN PER SPECIMEN AND PER GRAM IN *MEGANYCTIPHANES NORVEGICA* FROM DIFFERENT LOCALITIES

Specimens collected during November 1950

Locality	Date	No. of specimens	Av. wt. (mg)	Oil (%)	Vitamin A		Astaxanthin	
					i.u./spec.	i.u./g	µg/spec.	µg/g
Sandy Bank, Faeroe	6	91	80	3·9	1·3	16	5·1	65
Sandy Bank, Faeroe	6	225	100	4·1	1·5	16	5·4	55
Loch Fyne	21	93	85	3·2	1·3	15	4·8	56
Loch Fyne	21	92	62	4·6	1·0	17	5·8	93
Loch Fyne	21	238	66	4·0	1·2	18	4·5	68
Loch Fyne	21	174	55	5·1	1·1	20	5·1	93

TABLE III. OIL PER CENT AND VITAMIN A AND ASTAXANTHIN PER SPECIMEN AND PER GRAM IN *MEGANYCTIPHANES NORVEGICA* FROM DIFFERENT LOCALITIES

Specimens collected during August 1951

Locality	Date	No. of specimens	Av. wt. (mg)	Oil (%)	Vitamin A		Astaxanthin	
					i.u./spec.	i.u./g	µg/spec.	µg/g
63° 18′ N., 18° 39′ W.	29	33	66	2·9	0·5	7·4	3·1	46
Loch Fyne	16	164	55	1·2	0·6	11	2·2	40
Loch Fyne	16	210	80	2·4	0·9	11	2·8	35
63° 18′ N., 18° 39′ W.	29	14	222	2·9	1·7	7·8	9·3	42
Monaco	24	224	224	0·5	6·5	29	14	62
Loch Fyne	16	5	265	5·2	6·5	25	12	46
63° 18′ N., 18° 39′ W.	29	16	297	3·7	3·3	11	22	74
Loch Fyne	16	11	326	6·1	5·2	16	15	47
63° 18′ N., 18° 39′ W.	29	29	360	4·7	3·7	10	17	47
Loch Fyne	16	6	338	4·8	6·0	18	17	50

TABLE IV. OIL PER CENT AND VITAMIN A AND ASTAXANTHIN PER SPECIMEN AND PER GRAM IN *MEGANYCTIPHANES NORVEGICA* FROM DIFFERENT LOCALITIES

Specimens collected during January and February 1952

Locality	Date	No. of specimens	Av. wt. (mg)	Oil (%)	Vitamin A		Astaxanthin	
					i.u./spec.	i.u./g	µg/spec.	µg/g
Loch Fyne	16. i.	69	96	2·5	0·9	10	5·2	54
Monaco	29. i.	167	83	1·6	1·5	18	5·4	65
Monaco	29. i.	113	80	1·2	1·6	20	5·6	70
Monaco	29. i.	50	95	0·9	1·6	16	5·2	55
Loch Fyne	13. ii.	8	64	2·2	1·1	17	4·2	66
Loch Fyne	13. ii.	13	97	1·5	0·9	10	4·5	48
Loch Fyne	13. ii.	12	125	0·7	3·0	24	6·1	49
Loch Fyne	13. ii.	26	129	1·2	2·0	15	6·3	49
Monaco	12. ii.	150	79	1·5	1·5	20	5·9	74
Monaco	12. ii.	109	69	1·2	1·6	24	5·6	81
Monaco	12. ii.	50	94	2·0	1·7	18	5·8	62
Monaco	19. ii.	87	94	0·8	1·2	13	5·0	54
Monaco	19. ii.	63	101	1·0	1·2	12	5·7	56

Thysanoessa raschii (*M. Sars*)

We have so far analysed 190 groups of measured specimens of *T. raschii* and the range of vitamin A concentrations is shown in Fig. 2. The concentrations were much more widely distributed than in *Meganyctiphanes norvegica* although half the groups had concentrations between 10 and 45 i.u./g., with a weighted average for the whole series of 33 i.u./g. As in *M. norvegica*, very high concentrations were found only in groups of larvae (see Fisher *et al.*, 1954).

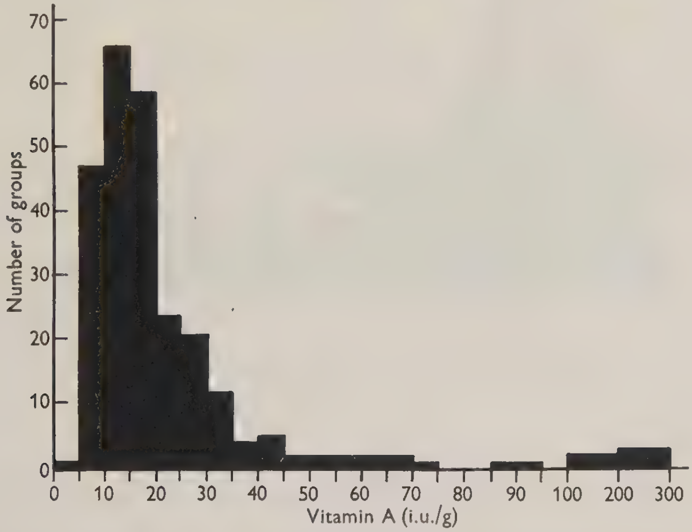

Fig. 1. Frequency distribution of concentration of vitamin A in 258 groups of *Meganyctiphanes norvegica*, containing from one to 359 specimens.

Thysanoessa inermis *Kröyer*

The results obtained from the analysis of specimens taken from the throat of a Blue Whale are given in Table I. The fat concentration was lower than in the samples of *Meganyctiphanes norvegica* collected from a Fin Whale at this station, and also lower than in free-swimming specimens of *Thysanoessa inermis* previously analysed by us (Fisher *et al.*, 1952). The concentrations of vitamin A and astaxanthin were of the same order as found in *Meganyctiphanes norvegica* from the whale and the vitamin A value was similar to that obtained from surface-swimming specimens of *Thysanoessa inermis* (Fisher *et al.*, 1952). The concentration of astaxanthin was only about half that of any of the free-swimming animals.

Thysanoessa gregaria *G. O. Sars*

A group of eight specimens, total weight 25 mg, separated into eyes and bodies, was analysed. The results appear in Table V. All the vitamin A was in the eyes, but there were only traces of carotenoid pigment present, insufficient to be determined, though behaving chromatographically like astaxanthin.

In this small weight of material there was also too little fat for an accurate determination by our technique.

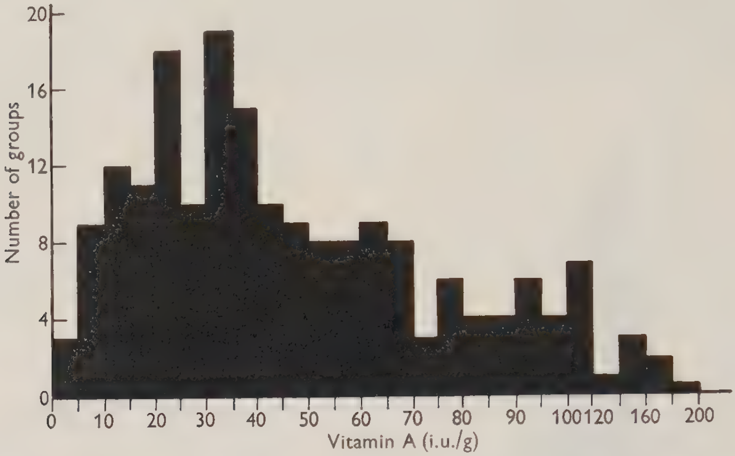

Fig. 2. Frequency distribution of concentration of vitamin A in 190 groups of *Thysanoessa raschii*, containing from one to 479 specimens.

Thysanoessa spinifera *Holmes*

There were nine specimens of this species with a total weight of 127 mg. Eyes and bodies were analysed separately and the results given in Table V show that vitamin A was exclusively in the eyes. The concentrations of fat and astaxanthin, which was the only carotenoid present, were of the order found in free-swimming animals of other species of *Thysanoessa*.

Thysanopoda acutifrons *Holt & Tattersall*

There were five specimens of this euphausiid in the group which weighed 160 mg, and bodies and eyes were analysed separately. The results in Table V show that vitamin A was not found in this species and that the concentrations of fat and astaxanthin were lower than we have found in other euphausiids.

Stylocheiron elongatum *G. O. Sars*

Four specimens of this species were analysed. Their total weight was 127 mg. Results of analyses appear in Table V. Vitamin A was present in a concentration similar to that in other euphausiids except *Thysanopoda acutifrons*. The concentration of fat was rather low compared with that of other species, but that of astaxanthin was much higher than usually found in euphausiids. No other carotenoids were observed.

Stylocheiron maximum *Hansen*

Only two animals of this species, with a total weight of 105 mg, were obtained. These were separated into eyes and bodies for analysis and the results are given in Table V. Vitamin A was again found only in the eyes and the concentration in the whole animals was very similar to that found in *S. elongatum*. Fat concentrations in the two species were also of the same order, but in *S. maximum* astaxanthin was found in lower concentration, resembling that in most other euphausiids.

TABLE V. OIL PER CENT AND VITAMIN A AND ASTAXANTHIN PER SPECIMEN AND PER GRAM IN SIX SPECIES OF EUPHAUSIIDS

Species	No. of specimens	Av. wt. (mg)	Oil (%)	Vitamin A		Astaxanthin	
				i.u./spec.	i.u./g	μg/spec.	μg/g
Thysanoessa gregaria (P)							
Eyes (pairs)	8	—	—	0·15	—	0	0
Bodies	8	3	—	0	0	0	0
Total	8	3	—	0·15	47	0	0
T. spinifera (P)							
Eyes (pairs)	9	—	—	0·14	—	0·4	—
Bodies	9	14	8·7	0	0	0·5	36
Total	9	14	8·7	0·14	9·4	0·9	63
Thysanopoda acutifrons (A)							
Eyes (pairs)	5	0·3	—	0	0	0	0
Bodies	5	32	0·4	0	0	0·7	21
Total	5	32	0·4	0	0	0·7	21
Stylocheiron elongatum (A)							
Eyes (pairs)	4	2	2·3	0·4	184	2·4	1090
Bodies	4	30	0·8	0	0	1·2	41
Total	4	32	0·9	0·4	13	3·6	113
S. maximum (P)							
Eyes (pairs)	2	7	14	0·7	97	2·6	365
Bodies	2	46	8·4	0	0	0	0
Total	2	53	9·0	0·7	13	2·6	50
Nematoscelis difficilis (P)							
Eyes (pairs)	40	2	5·2	0·3	184	0·5	314
Bodies	40	35	3·3	0·1	3	0·3	10
Total	40	37	3·4	0·4	11	0·8	23

A, specimens collected in North Atlantic on 2 October 1952 from R.R.S. *Discovery II*; P, specimens collected in North Pacific on 25 May 1953 from R.S. *Horizon*.

Nematoscelis difficilis *Hansen*

A group of forty specimens of *N. difficilis*, weighing 1463 mg, was divided into eyes and bodies which were analysed separately as in previous species. The results, given in Table V, show that this species contained vitamin A in a concentration of the usual euphausiid order, but only 75% of it in the eyes, contrasting with 90–100% normally found in other species. The concentration of astaxanthin, or its esters, was lower than in most species examined.

Euphausia superba *Dana*

The results for the various groups examined are shown together in Table VI. The vitamin A concentrations were of the same order in all the groups and all lower than in other euphausiids containing it. Although the conditions of storage of the *Discovery* samples were regarded as unfavourable, the vitamin A concentrations closely resembled those in the corresponding size-group (25–26 mm) of the *Balaena* 1953 samples, which were stored at a much lower temperature. It is suspected that the *Balaena* 1952 samples may have been regurgitated by a whale and, therefore, possibly partially digested. This would account for the rather lower concentration of vitamin A in the eyes and also for the higher concentration of astaxanthin in the bodies than in the free-swimming specimens collected in 1953.

Vitamin A concentrations in the 1953 size-groups increase with size up to the group of animals 31–32 mm long, just as we have found (Fisher *et al.*, 1954) in northern euphausiids, but there is an unaccountable drop in the larger groups.

The concentrations of astaxanthin in all the groups are closely similar and about one-third of those we have found in *Meganyctiphanes norvegica*. The proportion of this carotenoid in the eyes of *Euphausia superba* is, however, as high as in *Meganyctiphanes norvegica* (Fisher *et al.*, 1954).

Euphausia pacifica *Hansen*

Three groups of this species were collected; one consisting of 100 specimens, total weight 4·99 g, separated into eyes and bodies, was taken on 25 May 1953; and two groups, of 400 and 1001 specimens, weighing 3·42 and 16·5 g respectively, were taken on 26 May 1953. These two groups were preserved whole, without dissection. Analytical results are given in Table VII. The dissected specimens contained vitamin A, all in the eyes, and the concentration in these animals was as found in most other euphausiids. The whole specimens were smaller but there was a larger bulk of material. Even so, no trace of vitamin A was detected in them. Fat concentrations were the same in the first two groups and lower in the third. Astaxanthin was the only carotenoid present in all three groups in varying concentrations, all rather lower than usually found in, for example, *Meganyctiphanes norvegica*.

Study of Vitamin A Variations in Preserved Euphausiids

It would appear from the results shown in Table VII that vitamin A which might have been present in the eyes of the specimens of *Euphausia pacifica* preserved without previous dissection was destroyed by some substance in the bodies released by preservation. In order to verify this supposition the experiments recorded in Tables VIII and IX were done.

TABLE VI. OIL PER CENT AND VITAMIN A AND ASTAXANTHIN PER SPECIMEN AND PER GRAM IN *EUPHAUSIA SUPERBA*

Part examined	No. of specimens	Av. wt. (mg)	Oil (%)	Vitamin A		Astaxanthin	
				i.u./spec.	i.u./g	µg/spec.	µg/g
16. vii. 51. *Discovery II* station 2864							
Eyes (pairs)	197	1·4	12	0·6	437	2·3	1690
Bodies	197	195	2·3	0	0	2·1	11
Total	197	196	2·3	0·6	3·2	4·4	22
Early 1952. By net from *Balaena*. Antarctic							
Eyes (pairs)	148	0·7	5·0	0·02	34	0·2	307
Bodies*	—	—	1·4	—	1·3	—	33
Bodies + eyes	—	—	1·5	—	1·8	—	36
Early 1953. Engine room intake of *Balaena*. Antarctic							
Eyes (pairs)	148	3·3	8·4	0·9	263	2·5	753
Bodies	148	828	1·1	0	0	2·5	3·0
Total	148	831	1·2	0·9	1·4	5·0	6·0
Early 1953. By net from meat-carrying ship with *Balaena*. Antarctic							
Whole animals	47	87	4·2	0·3	3·7	0·9	11
(<22 mm long)							
Eyes (pairs)	14	1·4	3·2	0·7	508	0·9	653
Bodies	14	124	4·4	0	0	1·1	9·2
Total	14	125	4·1	0·7	5·5	2·0	16
(23–24 mm long)							
Eyes (pairs)	22	1·4	6·0	0·7	500	2·1	1508
Bodies	22	197	2·9	0	0	0·8	4·2
Total	22	198	2·9	0·7	3·4	2·9	15
(25–26 mm long)							
Eyes (pairs)	11	1·7	5·8	1·1	653	2·5	1475
Bodies	11	252	3·3	0	0	2·1	8·2
Total	11	254	3·3	1·1	4·4	4·6	18
(27–28 mm long)							
Eyes (pairs)	22	2·2	1·3	1·3	617	2·8	1288
Bodies	22	264	4·0	0	0	1·4	5·4
Total	22	266	4·0	1·3	5·0	4·2	16
(29–30 mm long)							
Eyes (pairs)	17	2·9	3·9	2·5	859	4·1	1411
Bodies	17	367	4·2	0	0	2·3	6·3
Total	17	370	4·2	2·5	6·7	6·4	17
(31–32 mm long)							
Eyes (pairs)	11	2·0	6·8	1·2	591	3·6	1784
Bodies	11	505	2·7	0	0	1·9	3·8
Total	11	507	2·7	1·2	2·3	5·5	11
(33–34 mm long)							
Eyes (pairs)	23	5·2	6·0	2·0	381	3·5	677
Bodies	23	524	2·2	0	0	5·1	9·7
Total	23	529	2·2	2·0	3·8	8·6	16
(35–36 mm long)							
Eyes (pairs)	15	3·6	4·5	1·5	425	4·2	1293
Bodies	15	548	2·5	0·5	0·9	3·2	5·9
Total	15	552	2·5	2·0	3·7	7·5	14
(37–38 mm long)							
Eyes (pairs)	19	3·2	4·3	1·4	453	4·4	1379
Bodies	19	661	1·1	0	0	5·8	8·7
Total	19	664	1·1	1·4	2·2	10	15
(39–40 mm long)							
Eyes (pairs)	20	3·1	3·5	1·8	584	3·4	1093
Bodies	20	587	1·9	0	0	9·7	17
Total	20	590	1·9	1·8	3·1	13	22
(> 40 mm long)							

* Somewhat macerated, hence uncountable.

In *Meganyctiphanes* (Table VIII), in all except two instances, the vitamin A content and concentration of the eyes and bodies separately was together greater than that of the corresponding group of whole animals. The two exceptions were the alcohol-preserved group of raw specimens which had been placed at a low temperature immediately after dissection and preservation on the ship, and might, therefore, be regarded as a control group and one of the alcohol-preserved groups of raw specimens stored for nearly 5 weeks in the deep-freeze. The content and concentration of astaxanthin were not consistently altered by the various methods of preservation or by the separation of eyes from bodies.

TABLE VII. OIL PER CENT AND VITAMIN A AND ASTAXANTHIN PER SPECIMEN AND PER GRAM IN *EUPHAUSIA PACIFICA*

Specimens collected in North Pacific on 25–26 May 1953

					Vitamin A		Astaxanthin	
Date	Part examined	No. of specimens	Av. wt. (mg)	Oil (%)	i.u./spec.	i.u./g	µg/spec.	µg/g
25. v.	Eyes (pairs)	100	1	13	0·5	515	0·7	683
	Bodies	100	49	1·9	0	0	0·4	7·7
	Total	100	50	2·2	0·5	11	1·1	22
26. v.	Whole specimens	400	9	2·2	0	0	0·3	35
26. v.	Whole specimens	1001	17	0·9	0	0	0·2	15

TABLE VIII. VITAMIN A AND ASTAXANTHIN PER SPECIMEN AND PER GRAM IN GROUPS OF *MEGANYCTIPHANES NORVEGICA* UNDER DIFFERENT CONDITIONS OF STORAGE

Groups of 25 specimens (length 23–25 mm) collected in Loch Fyne on 21–22 October 1953.

			Vitamin A		Astaxanthin	
Date tested	Preserved	Dissected	i.u./spec.	i.u./g.	µg/spec.	µg/g
30. x.	AF	W	3·1	15	10	48
30. x.	AF	E+B	2·7	13	8·4	39
23. xi.	AR	W	0·9	4·7	8·2	45
23. xi.	AR	E+B	2·4	10	14	57
27. xi.	AF	W	1·4	6·2	11	48
27. xi.	AF	E+B	2·3	10	14	60
27. xi.	AF	W	2·6	11	13	52
27. xi.	AF	E+B	2·3	10	8·9	40
9. xi.	BR	W	1·7	6·8	10	42
9. xi.	BR	E+B	3·1	14	6·4	29
23. xi.	BR	W	2·0	9·1	6·8	30
23. xi.	BR	E+B	2·6	12	6·3	30
27. xi.	BF	W	1·6	4·5	13	54
27. xi.	BF	E+B	2·8	13	13	59
27. xi.	BF	W	1·6	6·6	14	57
27. xi.	BF	E+B	4·0	18	13	61

A, raw specimens preserved in alcohol; B, boiled specimens preserved in alcohol; R, room temperature; F, deep-freeze; W, whole specimens; E+B, eyes and bodies analysed separately and results combined.

Although differences were found between dissected and whole animals in the vitamin A content and concentration of *Thysanoessa* (Table IX), they were not so marked as in *Meganyctiphanes*. The week-old alcohol-preserved raw specimens kept at a low temperature had a higher vitamin A content and concentration in whole than in dissected specimens. In the other groups the vitamin A concentrations were always higher in dissected than in whole animals. In all except one group the astaxanthin content and concentration were markedly higher in the dissected than in the whole specimens, in contrast to the results for *Meganyctiphanes* (Table VIII).

TABLE IX. VITAMIN A AND ASTAXANTHIN PER SPECIMEN AND PER GRAM IN GROUPS OF *THYSANOESSA RASCHII* UNDER DIFFERENT CONDITIONS OF STORAGE

Groups of 20 specimens (length 11–13 mm) collected in Loch Fyne on 21–22 October 1953.

			Vitamin A		Astaxanthin	
Date tested	Preserved	Dissected	i.u./spec.	i.u./g	μg/spec.	μg/g
30. x.	AF	W	0·7	37	0·9	47
30. x.	AF	E+B	0·5	25	1·1	56
25. xi.	AR	W	0·5	27	0·6	33
25. xi.	AR	E+B	1·0	59	1·5	89
27. xi.	AF	W	1·4	68	0·7	33
27. xi.	AF	E+B	1·5	75	1·0	51
27. xi.	AF	W	0·6	28	1·1	51
27. xi.	AF	E+B	0·9	58	1·4	90
9. xi.	BR	W	1·1	61	1·1	64
9. xi.	BR	E+B	1·6	69	1·6	66
25. xi.	BR	W	0·9	38	0·7	33
25. xi.	BR	E+B	1·0	42	0·5	20
27. xi.	BF	W	0·6	28	0·9	40
27. xi.	BF	E+B	0·8	38	1·2	57
27. xi.	BF	W	0·4	15	1·0	38
27. xi.	BF	E+B	0·4	22	1·1	54

A, raw specimens preserved in alcohol; B, boiled specimens preserved in alcohol; R, room temperature; F, deep-freeze; W, whole specimens; E+B, eyes and bodies analysed separately and results combined.

DISCUSSION

Comparison of the concentrations of vitamin A, astaxanthin and oil in *M. norvegica* collected from three different localities in August 1951 (Table III) shows that the specimens taken from the open sea were poorer in vitamin A but not in astaxanthin than those from more enclosed waters. Fat concentrations were similar in the two more northerly groups but much lower in the Mediterranean animals. We have previously pointed out (Fisher *et al.*, 1953) that the low fat content might be associated with the relative poverty in plankton of the Mediterranean (Bernard, 1938), but if that were so it is surprising that the reserves of vitamin A and astaxanthin accumulated by the euphausiids were not similarly reduced. A further comparison of the Loch Fyne and Monaco specimens collected in February 1952 (Table IV) supports the earlier evidence of little difference between them in vitamin A concentration, although in January the Monaco samples were richer than the only comparable Loch Fyne sample. Astaxanthin concentrations were again very similar in samples from both localities taken in both months. Fat concentrations in the January samples from Monaco were lower than in the Loch Fyne sample. The February samples from both places had similar fat concentrations. Samples of *M. norvegica* from Loch Fyne and The Faeroes area, two places relatively close together, differed little in their reserves of fat, vitamin A or astaxanthin (Table II).

According to Sheard (1953) some eighty-six species of the order Euphausiacea are recognized. In our studies of the vitamin A and carotenoid contents of animals of this order we have so far analysed eleven species, in nine of which vitamin A was found in high concentrations. The vitamin was absent from *Thysanopoda acutifrons*, but the amount of material analysed was small, although smaller weights of other species have been found to contain vitamin A. The eyes of *T. acutifrons* are, however, noticeably smaller than those of the other euphausiids we have examined and, since nearly all the vitamin A of euphausiids is found in the eyes, the two facts may be in some way associated, although Chun (1893) in his paper on euphausiid eyes makes no mention of any peculiarities in the genus *Thysanopoda*. Further investigation of the species with a larger amount of material is clearly indicated. The concentration of vitamin A in the samples of *Euphausia superba* we have examined was lower than in the other species of Euphausiacea containing it, but it is possible that some destruction of the vitamin A may have occurred during the passage of these specimens from the Antarctic through the tropics to this country. Even so, the concentration of vitamin A in this species is still higher than in any of the decapod Crustacea we have examined, the richest example of these being *Acanthephyra purpurea* Milne-Edwards. The average values for vitamin A concentrations of the euphausiids we have studied are compared in Table X, which shows clearly how much richer in vitamin A they all, with one exception, are than other Eucarida so far examined by us.

In view of their unique richness in vitamin A among the Crustacea it may be profitable to discuss the affinities of the Euphausiacea. In the two most recent accounts of the order (Banner, 1950; Sheard, 1953) it has been placed with the order Decapoda in the series Eucarida, division Eumalacostraca of the subclass Malacostraca. Vitamin A is present in most of the Decapoda we have studied (Kon, 1954), but in much lower concentrations (see Table X) than in the euphausiids. We have learnt from Dr I. Gordon of the British Museum that the taxonomic position of the order Euphausiacea is not yet definitely established. She informs us that her views correspond to those of the late Dr S. W. Kemp who believed, with Calman (1910), that they should be more closely associated with the Penaeidea, themselves a suborder of the Decapoda.

Comparative biochemistry has provided support for morphological evidence used in taxonomy in the work of Baldwin and his colleagues on muscle phosphagens in the animal kingdom (see Baldwin, 1937). It is possible that we may have in vitamin A, or the ability to accumulate it, a similar taxonomic indicator related to variations in the enzyme systems of the different groups of Crustacea. We have shown (Fisher *et al.*, 1953) that the Penaeidea have similar concentrations of vitamin A to those of other Decapoda, whereas the Euphausiacea are much richer, so that, on this evidence, the two groups are not so closely related as are the Penaeidea with the Decapoda.

TABLE X. DESCENDING ORDER OF AVERAGE VALUES FOR VITAMIN A CONCENTRATIONS IN EUPHAUSIACEA, AND IN DECAPOD RICHEST IN VITAMIN A

	Vitamin A (i.u./g)	Localities	No. of groups
Thysanoessa gregaria	47	P	1
T. raschii	33	A	190
T. inermis	16	A	3
Meganyctiphanes norvegica	15	A, M, N	258
Stylocheiron elongatum	13	A	1
S. maximum	13	P	1
Euphausia pacifica	11	P	1
Nematoscelis difficilis	11	P	1
Thysanoessa spinifera	9·4	P	1
Euphausia superba	3·1	An.	13
Acanthephyra purpurea	2·7	A	1

Average concentrations have been weighted for the numbers of specimens in each group. Values for free-swimming specimens only have been taken. A, Atlantic; An., Antarctic; M, Mediterranean; P, Pacific; N, Norwegian Sea. Vitamin A absent from *Thysanopoda acutifrons*.

The Euphausiacea live in similar habitats to the Mysidacea, with which they were for long classified as the Schizopoda. We have analysed four Mediterranean species of mysids (Fisher *et al.*, 1953) and *Hemimysis lamornae*, obtained from the aquarium tanks of the Plymouth laboratory, and they all lacked vitamin A. More recently we were supplied by Dr Dexter S. Haven of the Virginia Fisheries Laboratory with a large sample of *Neomysis americana* (S. I. Smith), collected from the York River, Virginia, on 9 February 1954, and found in them a vitamin A concentration of 1·9 i.u./g. Nevertheless, the evidence so far indicates that the euphausiids are biochemically very different from other Crustacea, including the Penaeidea, considered to be their nearest relatives, and the Mysidacea which are their nearest neighbours in the sea.

Einarsson (1945), Banner (1950) and Sheard (1953), who have all written at length on the Euphausiacea, are unanimous in stressing the importance of these animals in the economy of the seas. Sheard ascribes to them two general roles: 'the first being that of intercepting organic nutrient material' derived from the breakdown of phytoplankton, for example, the faecal pellets of grazing herbivores, 'and of returning it directly to the carnivore population'; 'the second (together with the pelagic larvae of bottom-living invertebrates) being that of delivering some products of the bottom cycle of organic production to the pelagic (nutrient) cycle'. The second function would be achieved by the detrital feeders. Sheard further points out that 'this dual buffer and transference role of Euphausiacea', may explain 'the apparent excess of production of carnivores, when measured against primary nutrient values and phytoplankton production in subtropical and tropical seas'.

More detailed information about the role of euphausiids as food organisms for many whales and fishes has been provided by Einarsson (1945). He stated that the Blue Whale (*Balaenoptera sibbaldi*) lives exclusively on euphausiids, *Thysanoessa inermis* and *Meganyctiphanes norvegica* in arctic and *Euphausia superba* in antarctic seas. The Fin Whale (*Balaenoptera physalus*) also feeds largely on these species. We have already (Fisher *et al.*, 1952) drawn attention to the direct passage of the rich vitamin A supplies of these euphausiids to the stores of the whale.

Sperm Whales (*Physeter catodon*) feed on cuttlefishes and squids, in which vitamin A has been found in good concentrations in the liver (Brachi, 1953; Kon, 1954). These cephalopods live mainly on Crustacea and since ten-armed forms, important as whale food, are pelagic in habit they probably feed largely on free-swimming species, among which, by virtue of their enormous numbers and swarming habits, the euphausiids must rank as of considerable importance. Hjort & Ruud (1929) definitely stated that euphausiids form the food of the squid, *Gonatus fabricii*, which is eaten by the Bottle-nose Whales (*Hyperoodon rostratus*). It seems likely, therefore, that euphausiids are directly or indirectly the main source of vitamin A for all species of commercially valuable whales.

Copepods are the most important item in the diet of the herring (*Clupea harengus*), but they contain no more than traces of vitamin A (Fisher *et al.*, 1952). Recent work in this laboratory, so far unpublished, has shown that herring-liver oil may contain vitamin A in concentrations up to 60,000 i.u./g, and that the concentration is also high in the intestine. Einarsson (1945) in a review of the literature points out that, next to copepods, euphausiids are the food animals most favoured by the herring and, in some localities, they may form the bulk of the diet, as for example in the Skagerrak, where Poulsen (1926) reported that in May herring were feeding almost exclusively on *Meganyctiphanes norvegica*. This euphausiid is eaten by herring in the Clyde Sea area to the extent that the stomach becomes distended, the condition being described by fishermen, who reject these fish, as 'gut-poke'. Moore (1898) described *M. norvegica* as the most important food of the herring off the Atlantic coast of America.

A detailed study of the food of the cod (*Gadus callarias*) was made by Brown & Cheng (1946), who concluded that, for cod from the Bear Island and Spitzbergen Bank areas, pelagic Crustacea, especially *Thysanoessa inermis*, are the most important component from February to August, and young herring from November to December. Capelin (*Mallotus villosus*) is the principal food of the cod off the Murman coast and sand-eels (*Ammodytes* spp.) off Andanes and Iceland. The capelin feeds on *Thysanoessa inermis*, according to Saemundsson (1937). Wiborg (1949) stated that the main food of cod of the O-II-groups from deep water along the coast of northern Norway consisted of euphausiids, especially *T. inermis* and *Meganyctiphanes norvegica*. Thus euphausiid vitamin A also seems to be the source of the rich reserves found in cod-liver oil.

Halibut-liver oil is one of the richest natural sources of vitamin A, and Lovern & Sharp (1933) studied the food of this fish in an attempt to find the source of its reserves. The torsk (*Brosmius brosme*) was the commonest species eaten and torsk-liver oil proved richer in vitamin A than most cod-liver oils. Poulsen (1926) reported that torsk was one of the species to be found feeding on *Meganyctiphanes* in the Skagerrak. McIntyre (1952) more recently reported that halibut around Iceland and the Faeroes feed mainly on *Sebastes marinus*, together with other fish, especially herring, decapod Crustacea, especially *Nephrops norvegicus* and some cephalopods. Lovern, Edisbury & Morton (1933) drew attention to the wide variations between the potencies of individual samples of halibut-liver oil and these variations may be associated directly with the type of food eaten, some fish feeding consistently on food richer in vitamin A than that eaten by others. Euphausiids may, therefore, be the initial source of the vitamin A in halibut liver.

Saemundsson (1926) and Macdonald (1927) reported that the saithe (*Gadus virens*), which is the basis of important fisheries, also feeds on euphausiids. Lovern *et al.* (1933) mentioned that saithe-liver oil is of similar vitamin A potency to that of the cod and hake. The hake itself (*Merluccius vulgaris*) was reported by Hickling (1927) to feed selectively on *Meganyctiphanes*, and this euphausiid has been observed by us in the stomachs of hake caught in Loch Fyne.

An example of a number of fish listed by Lovern *et al.* (1933) as being poorer in vitamin A than the cod is the haddock (*Gadus aeglefinus*). This species has been recorded as feeding occasionally on euphausiids in Icelandic waters (Saemundsson, 1937), around Bear Island (Robertson, 1932) and in the Barents Sea (Zatsepin, 1939). In the Skagerrak, Poulsen (1926) found haddock feeding in May almost exclusively on *Meganyctiphanes norvegica*. Einarsson (1945) considers, however, that euphausiids are not of major importance as food for this fish and they were not found in any of the stomachs of haddock, from Icelandic waters or the Murman coast, examined by Brown & Cheng (1946). Einarsson (1945) names several other economically important species of fish which feed on euphausiids, and the foregoing review emphasizes the importance of this order of Crustacea not only because of the two more general roles mentioned by Sheard (1953) but also in the more specific part they play as a source of the vitamin A stores of marine vertebrates. We have previously (Fisher *et al.*, 1954) discussed, and are still investigating, possible sources of euphausiid vitamin A, and it may be that the animals of this order possess an enzyme equipment that permits them to break down carotenoids other than those normally regarded as vitamin A precursors and in such a way that vitamin A appears as an end-product in very large amounts which are accumulated in the eyes. These reserves are passed on directly from the euphausiids to their cephalopod and vertebrate predators which store the vitamin in their livers, accumulating it throughout life (Macpherson, 1933), only a small proportion being utilized.

It certainly seems that the euphausiids form a unique reservoir in the sea of vitamin A which they accumulate from an unusual source (see Fisher *et al.*, 1954). The vitamin itself exhibits certain peculiar properties, both in its discrepant physicochemical and biological potencies (Fisher *et al.*, 1952) and in its apparent destruction under certain conditions of storage now reported. We are making an intensive biochemical study of these phenomena and also seeking material to extend our observations on the one hand to other species of Euphausiacea to determine whether high concentrations of vitamin A are a universal feature of the order, and on the other to other Crustacea to search for a species as rich in vitamin A as any of the Euphausiacea.

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SUMMARY

Eleven species of Euphausiacea, namely, *Meganyctiphanes norvegica*, *Thysanoessa raschii*, *T. inermis*, *T. gregaria*, *T. spinifera*, *Thysanopoda acutifrons*, *Euphausia superba*, *E. pacifica*, *Nematoscelis difficilis*, *Stylocheiron maximum* and *S. elongatum* have been analysed for fat, vitamin A and carotenoids. Vitamin A was present in all species except *Thysanopoda acutifrons*, but in *Euphausia superba* the concentrations were of a lower order although still higher than found by us so far in any decapod crustacean.

Astaxanthin, or its esters, was the only carotenoid found in all the species of euphausiids examined.

Groups of *Meganyctiphanes norvegica* from enclosed waters, namely, Loch Fyne and the Mediterranean Sea, had higher reserves of vitamin A than those from Atlantic waters. The fat concentrations of the Mediterranean specimens were lower than those of animals from more northerly waters but the vitamin A or astaxanthin concentrations of the two groups were similar.

In *Euphausia pacifica*, *Meganyctiphanes norvegica* and *Thysanoessa raschii*, the vitamin A content of animals separated into eyes and bodies before preservation and analysis was higher than that of animals left whole. The possible presence in the bodies of a substance causing destruction of vitamin A is being investigated.

The role of euphausiids is discussed as a vital link in the passage of nutrient substances from detritus, phytoplankton and zooplankton to marine vertebrates, with particular reference to the possible conversion by these Crustacea of carotenoids to vitamin A which they accumulate in their eyes.

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THE OCCURRENCE AT PLYMOUTH OF THE OPISTHOBRANCH *AKERA BULLATA*, WITH NOTES ON ITS HABITS AND RELATIONSHIPS

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(Plate I and Text-figs. 1 and 2)

INTRODUCTION AND ECOLOGY

Akera bullata O. F. Müller is a tectibranch mollusc having a coiled external shell and a foot with lateral extensions used for swimming. At certain times of the year it swims actively, as was observed by Donovan, who writes that it 'sports in its watery element with all the liveliness of a butterfly, and formed a pleasing object when kept alive in a glass of sea water' (quoted by Montagu, 1803). Although sometimes abundant locally, it is not a mollusc often taken by the collector, even though it is a conspicuous object when swimming.

The first record of this species from Plymouth was the discovery of large numbers in a sea-water tank in the Naval Dockyard at Devonport, in April 1954. It has been recorded from a number of places in the British Isles, and ranges from Norway to the Mediterranean (Forbes & Hanley, 1853).

Two variants of the original species have been described: var. *nana* Jeffreys is a small form which was dredged in the Shetlands, and var. *farrani* Norman, a giant form from a locality in Ireland (Norman, 1890, p. 68). Since these so-called varieties apparently differ only in size, they would seem to be of doubtful validity. The specimens taken at Devonport were the normal form described by Müller, and listed as *A. bullata bullata* Müller by Winckworth (1932).

The specimens were found in an oval concrete tank, some 33·5 m long, 23 m broad, and 2·5 m deep. Water is continually drawn from the tank for use in the Dockyard, the level dropping perhaps 20–30 cm during the day. The tank is topped up daily with water drawn from the lower part of the Tamar estuary, opposite St John's Lake. The tank is some distance above sea level, and about a quarter of a mile from the river.

The sea-water intake is about 2 miles from the mouth of the Tamar, and at this point the surface salinity varies around 30‰ (Milne, 1938, p. 538). A single sample from the tank on 14 April 1954 had a salinity of 32·95‰

(density 1·02470 at 13·6° C), the temperature being 11·6° C. Salinity variations must be less sudden than in the estuary, since only a small proportion of the total volume is renewed daily.

The vertical sides of the tank were covered with a thick growth of green algae, mainly *Ulva*, and in the summer of 1954 there was also much filamentous green alga, both at the bottom and floating on the surface. There was a thin layer of mud on the bottom, but the water was usually quite clear, except just after pumping.

Apart from *Akera* the fauna was sparse, a few gobies, mysids, amphipods, and ascidians being taken. There were also two or three medium-sized fish, possibly pollack (*Gadus pollachius* L.). Apart from the abundance of algal food, *Akera* may have been favoured by the absence of predators. Although apparently distasteful to some fish, it has been found in the stomach of the flounder (Jeffreys, 1867, p. 432).

The outflow of water from the tank is evidently not so great as to sweep away considerable numbers of *Akera* larvae, as young specimens spawned in the tank were abundant in the summer of 1954.

The swimming habit of this animal has often been noted, and a detailed description of swimming movements is given below. On the first visit, on 14 April 1954, both adults and young were swimming in fair numbers, although there were always many more crawling on the sides and bottom of the tank. Since most individuals would swim after capture, it is fairly safe to say that at that time all, or nearly all, those in the tank were swimming intermittently. In the laboratory, adults would sometimes swim for periods of half an hour or more, and the young seemed capable of swimming for even longer periods. Swimming may be initiated by disturbing the animals, but individuals also swim spontaneously from time to time.

Swimming mainly takes place in the spring, which is the peak spawning period (cp. Guiart, 1901, p. 47). In April 1954 adults were copulating and spawning in the laboratory, and much spawn was found in the Devonport tank. *Akera* appears to have an extended breeding period, as a little spawn was found as late as 13 July, and small specimens, presumably spawned earlier in the year, were common in April. Thorson (1946) records that in Denmark egg-masses are found throughout the summer, indicating a rather later spawning season than at Plymouth.

On 29 April fewer adults and young were swimming than on the 14th. On the next visit, on 17 June, many young were crawling over the algae, but adults were very rare, and no *Akera* were seen swimming in spite of good visibility in the tank. Some of the young taken on this date did swim in a plunger jar in the laboratory, however, a few days later. On 13 July there were still none swimming, and young stages again predominated.

Observations are being continued, but the evidence so far collected indicates that the species is annual, growing to maturity and spawning after a year, after which the majority die. Swimming, both of adults and young, is more or less confined to the spring, and may perhaps be correlated with the breeding season. *Akera* may occasionally swim in the summer.

Akera normally lives on mud flats, among weeds or *Zostera*, but none have been recorded from Plymouth, nor in the Yealm and Salcombe estuaries (Marine Biological Association, 1931), where conditions would appear to be suitable. It is not recorded from Torbay (Jukes-Browne, 1910) or the Exe estuary (Allen & Todd, 1902, and observations by N. A. H. during 1946–47). The nearest records are from Falmouth and Helford, 40 miles to the west (Clark, 1906, and personal communication from Mr R. Baird, who has found it in the Fal in recent years). It has been taken 70 miles to the eastward in the Fleet, Dorset, by Mr G. M. Spooner, and at Roscoff, 100 miles to the south, across the English Channel (Station Biologique de Roscoff, 1951). One or more specimens were evidently introduced to the tank via the inflow pipe, but there is no evidence as to their origin.

EXTERNAL FORM AND SWIMMING

A description of the external features of *Akera bullata* has already been provided by Guiart (1901), showing the division of the body into two regions—a smaller, dorso-ventrally flattened head, connected by a narrow 'neck' to a larger, ovoid visceral mass. The spirally coiled shell is yellowish brown in colour and encloses the visceral mass and the mantle. The features of the shell are well described by Jeffreys (1867) and by Forbes & Hanley (1853). In the sedentary posture, the neck and most of the visceral mass of the animal are concealed by the upgrowth of a pair of large, thin flaps, the parapodia, or 'wings' which are expansions of the ventral surface upon which *Akera* creeps. At their widest parts the wings overlap upon the dorsal surface, surrounding most of the body, so that the animal, when crawling fully extended, has a narrowly conical appearance, broadening behind, where the visceral hump and shell protrude from the parapodia. A long white filament, forming a grooved tentacle produced from the pallial margin, trails behind. In the crawling animal the right parapodium seems invariably to lie above the left, but when the parapodia come to rest between successive swimming movements, this arrangement is reversed, and it is the edge of the left parapodium which lies uppermost. *A. bullata* shows on the parapodia and ventral surface a ground colour of ashen brown or grey, closely spotted and blotched, as in an *Aplysia*, with greyish white. Inside the parapodia, and on the narrow 'neck' the colour is lighter and the head is streaked longitudinally with a series of broken lines of dark brown or black.

In crawling—which is its most frequent movement—*Akera* proceeds on the flattened ventral surface which is continuous with the parapodia. This surface is in no way marked off as a distinct sole, and is pigmented in the same way as the parapodia themselves. It is richly supplied with mucous glands and the animal appears often to secrete about itself a tube of slime, from which it may then emerge by crawling out in front. At times the head and the visceral mass may be almost completely retracted within the cloak formed by the parapodia, and the animal sits motionless, taking on a compact flask-shape.

The swimming movements—when first seen—come as a surprise (see Text-fig. 1). They begin by the opening out of the parapodia, so as partly to display the body between them. The margins are then lifted up like a skirt and stand out freely from the body, while the anterior end of the animal rises first from the ground, so that the animal achieves an upright position, and sits making one or two tentative openings of the parapodia. The visceral mass can now be clearly seen below, like the clapper of a bell, as the parapodia are finally widely extended and the skirt is lifted high. As the edges of wings disengage the dorsal surface is also uncovered, and the skirt extends at right angles to the body, in the same plane as the creeping surface of the foot. The creeping surface is now presented uppermost, and forms a more rigid tract, terminating behind in a permanent point, while round it the flaps of the parapodia form a heart-shaped shield, with the edges slightly upcurved.

The skirt is now rapidly lowered, and its contraction against the water beneath it provides the motive power by which the animal leaves the ground and is lifted through the water. As the edges of the parapodia again come together, they enwrap the dorsal surface, the left one this time uppermost. Almost at once they are opened again and spread flat, to contract downwards in another effector stroke which carries the animal further upwards. After each swimming stroke, the animal loses way by sinking a short distance from the weight of the visceral hump, before the next movement of the wings carries it upwards in a renewed spurt. An average of forty-five strokes a minute was reckoned for one specimen at normal sea temperature. The movements have an extraordinary lightness and grace, and an impression of the swimming animal may be gained from the photographs (Pl. I) made by Dr D. P. Wilson in the Plymouth laboratory.

Digestive System

Since the descriptions of the alimentary canal given by Bergh (1900) and Guiart (1901) are short and often inaccurate, an opportunity was taken to re-examine the gut, using living material and paying closer attention to functional aspects. In almost all its features the alimentary canal of *Akera* is entirely aplysioid, and the same is true of the mode of life. *Akera* is a grazer on green algae such as *Ulva*, or a deposit feeder on material already comminuted. The buccal mass does not need separate description from that of *Aplysia*, but a figure of the radula is provided here (Text-fig. 2C). Each tooth row has a series of teeth typical of the grazing and raking form of radula seen in Aplysiomorpha. The radula resembles much less that of the Bullomorpha, whether the herbivorous forms or the much more modified carnivores. Into

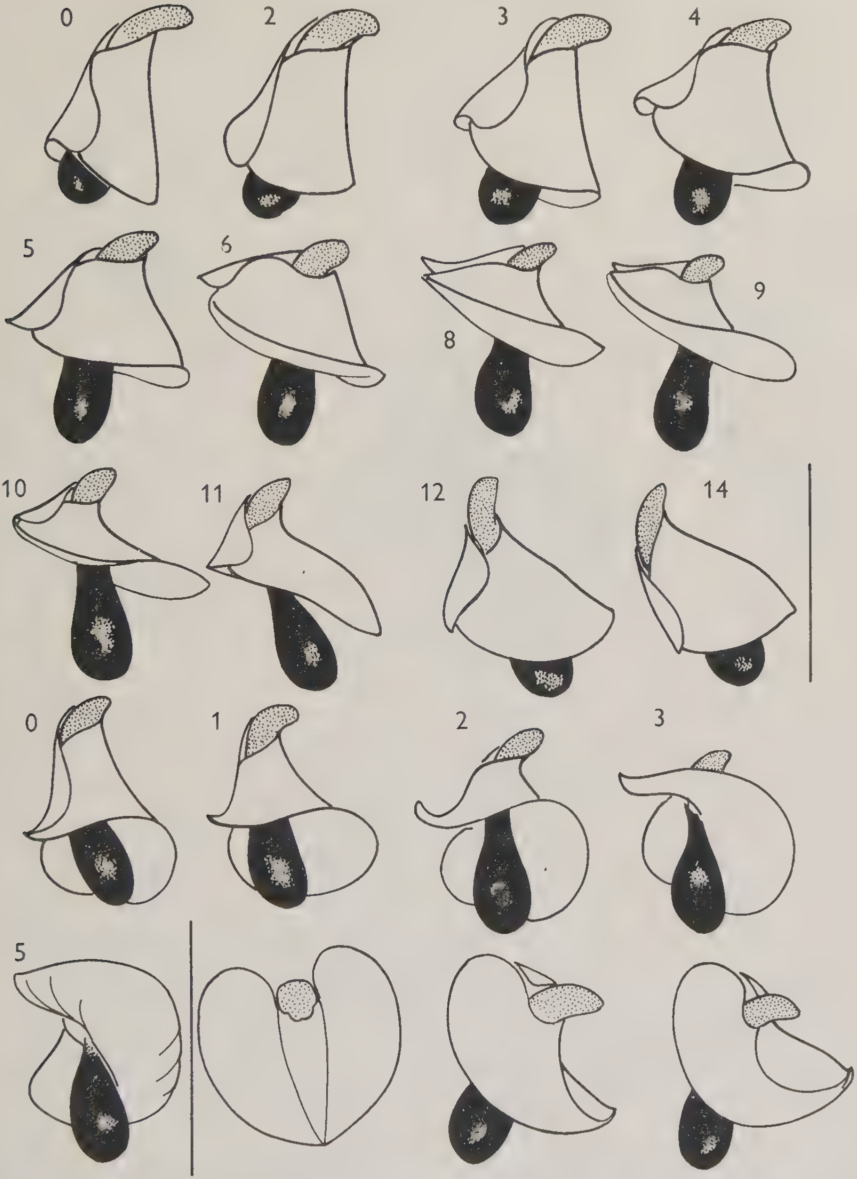

Fig. 1. Outline diagrams to show the positions of the parapodia at successive stages during the swimming movements. The drawings are constructed from a cine-film record and those of the first three rows comprise a continuous sequence, with the time intervals in sixteenths of a second shown. Those of the fourth row and the first of the fifth row form another series, and the three final drawings are selected to show further views of typical swimming postures.

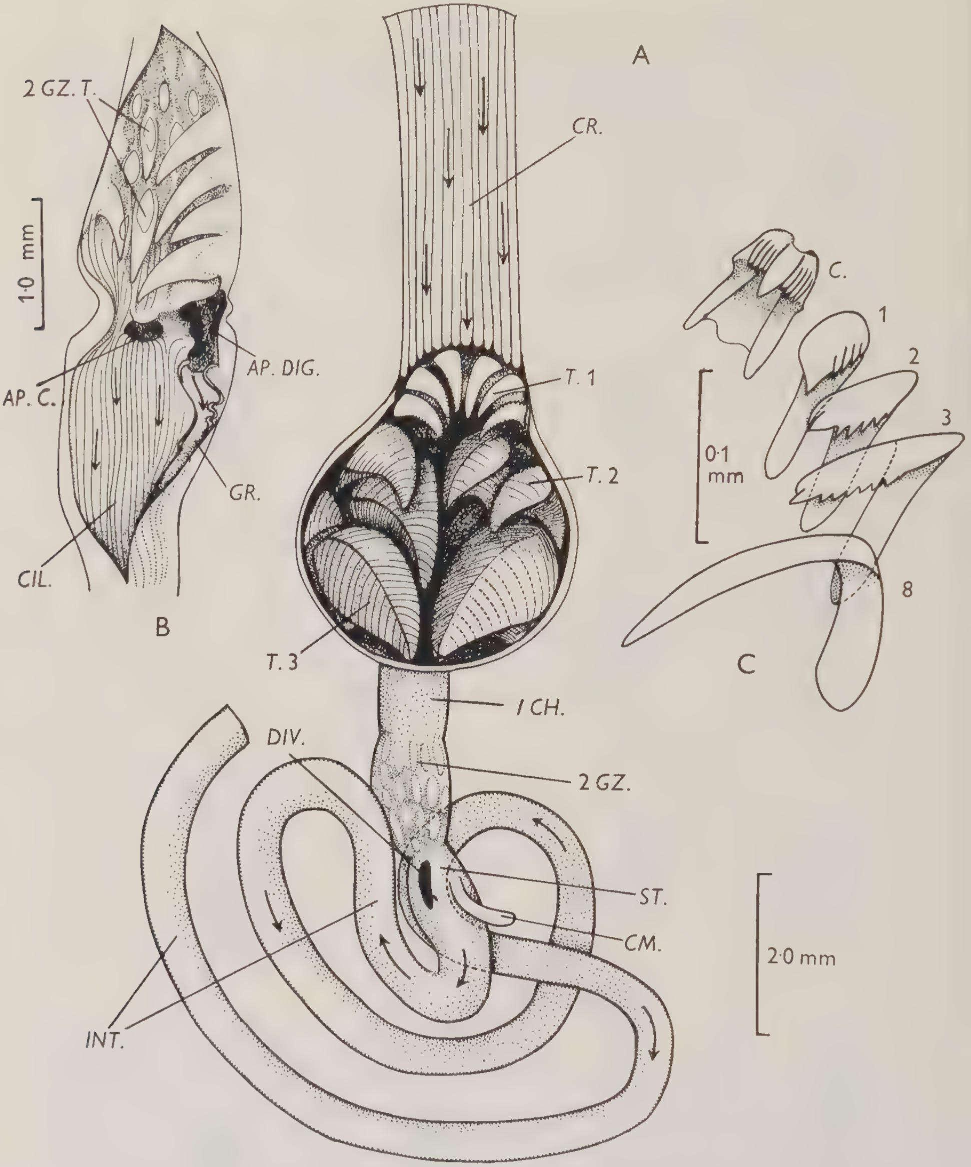

Fig. 2. A, general view of the alimentary canal removed from the animal with the buccal mass omitted, and the crop and first gizzard opened to illustrate their internal structure. B, more detailed view of the second gizzard and the stomach, dissected to show internal structure. C, central (*C*) and four of the marginal teeth (nos. 1, 2, 3 and 8) of the most posterior tooth row of the radula. *AP. C.*, aperture of the gastric caecum; *AP. DIG.*, opening of the digestive diverticulum into the stomach; *CIL.*, ciliated folds leading from the stomach into the intestine; *CM.*, gastric caecum; *CR.*, crop; *DIV.*, position of digestive diverticulum; *GR.*, groove leading into the intestine from the digestive diverticulum; 2 *GZ.*, second gizzard; 2 *GZ. T.*, teeth of the second gizzard; *I CH.*, intermediate chamber between the first and second gizzards; *INT.*, intestine; *ST.*, stomach; *T.* 1, *T.* 2; *T.* 3, teeth of the first, second and third tiers in the first gizzard.

the buccal mass open a pair of long, strap-like salivary glands, with short tubular ducts. They are orange yellow in the living animal, and their histology resembles that of *Aplysia*, as described by Howells (1942).

The next regions of the gut are contained in the narrow, neck-like part of the body, and comprise a long, very distensible crop, and a large first gizzard, both of which are oesophageal. The crop is thin-walled and is thrown into a large number of fine longitudinal ridges. It is translucent white in colour, and the contained food—fine detritus and roughly chopped-up filaments of algae—shows through its wall. The shape of the crop varies greatly, according to the position of food passing through it. It is capable of very strong peristaltic movements, so that temporary constrictions and bulges are likely to appear along its course. At times it appears like a fat sac, at others it is a narrow tube of uniform width. As well as being muscular and peristaltic, the crop is strongly ciliated, and—apart from the intestine—it is the only part of the gut where cilia appear to play an important role. All beat strongly in the same direction, along the ridges and furrows towards the first gizzard.

The first gizzard is pyriform in shape, and extremely firm-walled (see Text-fig. 2A). At its narrow anterior end the ciliated folds of the crop end abruptly, and a food string containing coarse pieces of algae and debris passes into the mill formed by the series of gizzard teeth. These teeth are arranged in three tiers, the largest ones comprising a set of four at the posterior end of the gizzard. These four are massive and broad-based, each consisting of a thick shell of cuticle applied to a strong boss of the epithelium. They are roughly pyramidal in shape, but slightly beaked so that their blunt-pointed tips, which meet at the centre of the gizzard are directly backwards towards the exit. The next tier of teeth are also four in number, slightly smaller and alternating with the posterior teeth. Their tips are sharper and much more strongly beaked, directed backwards. The teeth of the most anterior tier, immediately inside the entrance to the gizzard comprise a circlet of six to eight. They are small and slender, and form a strainer through which the food passes before being engaged and crushed between the tips of the larger teeth.

The food leaving the gizzard forms a soft, greyish green cord, much finer in consistency than when it was received from the crop. Behind the gizzard lies a smaller region of the gut, narrow and not toothed, forming an intermediate chamber by which the first gizzard communicates with the second gizzard. This intermediate chamber has a longitudinally ridged wall, and is contractile, though not strongly muscular. So far as could be made out from living material, it is not ciliated. Behind it the gut opens through a 'diaphragm' of muscle and connective tissue, by which the organs lying within the head and 'neck' are separated from those of the visceral mass. Behind the diaphragm the second gizzard opens by a slight constriction from the intermediate chamber. The second gizzard is a great deal smaller than the first, and its walls are less muscular, though still strongly contractile. The teeth are here much smaller, the larger ones long and narrow and pointed backwards. They are hardly at all rigid, being for the most part soft and flexible to the touch. They fill most of the gizzard and form a series of papillae, serving not for the attrition of food but for filtering the stream of particles that is admitted to the stomach. These teeth are arranged in seven or eight longitudinal rows and their oboid bases are very clear externally, through the thin wall of the gizzard. One specimen had twenty-five, most of them small and short.

Text-fig. 2B illustrates the internal structure of the second gizzard, and its relation to the stomach, which properly speaking forms that part of the gut lying immediately behind the gizzard. It receives a single digestive diverticulum, and is equipped with a short, finger-shaped caecum. It is marked off in front by a constriction from the second gizzard, and merges gradually behind into the intestine. The walls of the stomach are ciliated and thrown into fine longitudinal folds, which continue backwards from the tooth-bearing folds of the second gizzard and run on into the intestine. Some of these folds lead out from the mouth of the caecum, which is ciliated internally and in most ways resembles that of *Aplysia*. The function of the caecum appears to be to fashion segments of the faecal string which pass from its opening directly into the intestine where they merge into a continuous rope. The opening of the caecum is connected by a shallow channel with the mouth of the digestive diverticulum, which lies a little to the side of it, and immediately behind the largest (posterior) tooth of the second gizzard. Leading backwards through the stomach from the diverticulum is a broad gutter, lined with strongly beating cilia, which enters the intestine and there loses its identity. Of the sorting area, gastric shield, and style sac, represented in bullomorphs (see Fretter, 1939; Fretter & Graham, 1954) nothing remains in *Akera*.

The intestine describes a double loop, turning backwards after leaving the stomach, and running through the digestive gland to come to the surface on the right side. It then curves back again, running backwards above the stomach, around the visceral mass to the left. It finally runs forward and towards the mid-line as the rectum. The intestine is smooth-walled and finely ciliated, and carries a rope of fine, well-comminuted faeces. No peristaltic contractions could be observed, and the faecal string shows little sign of compression, or breaking into pellets. It issues free of the mantle cavity, breaking away from the anus at the edge of the mantle in large, irregular, coils.

Relationships

In the classification currently in use—that of Thiele (1931) which has been adopted by Hoffmann (1938)—*Akera* is placed within the suborder Cephalaspidea (Bullomorpha) of the order Pleurocoela (Tectibranchia), in the Akeratidae.[1] Two subfamilies are recognized by Thiele, namely the Akeratinae, containing the single genus *Akera*, and the Cylindrobullinae. Pelseneer (1906) had previously placed the Akeratidae within the Bullomorpha, thereby reversing the decision of Guiart (1901) who had put forward a number of reasons for considering these molluscs as more properly aplysiomorphs. From a further study of the structure of *Akera bullata*, it becomes clear that Guiart was right, and that *Akera* has many more characters in common with the Anaspidae (Aplysiomorpha) than with the Cephalaspidea (Bullomorpha). Of the features regarded by Pelseneer and later writers as diagnostic of the Bullomorpha, the following are the chief:

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[1] The family name is correctly formed as Akeratidae, as in Pelseneer (1906), not Akeridae, as cited by Thiele.

(i) The shell and pallial cavity are well developed and the gill is wholly or partly contained within the pallial cavity.

(ii) The head is devoid of apparent tentacles and is modified to form a disk-shaped cephalic shield, used for digging into the sand.

(iii) The parapodia are continuous with the ventral sole of the foot.

(iv) The gizzard is either lacking, or single, with relatively few, sometimes calcareous plates.

(v) The visceral commissure is usually long.

In (ii) and in (iv) *Akera* is obviously unlike the bullomorphs. Although the eyes are reduced, and obvious tentacles are lost, the head remains unspecialized and with none of the modifications associated with the habit of burrowing beneath the soil. In the structure of the gut—and this is a region that has taken on distinctive specializations in all aplysiomorphs—*Akera* has already been shown to have all aplysioid features. There is a large first gizzard, a smaller second gizzard, both with numerous teeth, and a small gastric caecum. Typical bullomorphs have none of these (see Fretter, 1939). The genitalia of *Akera* have not been investigated during this work, since they have already been dealt with in a comprehensive study by the late Dr Hilda Lloyd, which Dr Vera Fretter is preparing for publication. Dr Fretter has kindly allowed us to state that the reproductive system of *Akera* conforms in almost all respects to that of the aplysioids and has no distinctively bullomorph features. The mode of copulation with the formation of a chain of six or more individuals and the nature of the gelatinous egg-strings are also aplysioid features. The shell of the free-swimming veliger (see Thorson, 1946) has the initial sinistral twist detectable in Bullomorpha but almost suppressed in the other aplysiomorphs.

In addition, the mantle cavity in *Akera* possesses the two typically aplysioid glands, an opaline gland occupying its floor, and a purple gland located in the roof, and secreting, as in *Aplysia*, a purple-coloured fluid. Finally, as completing the list of aplysioid resemblances, Dr G. Y. Kennedy and Dr H. G. Vevers, in a forthcoming paper, will report similarities between *Aplysia* and *Akera* in the possession of the pigment Uroporphyrin I in the integument.

Pelseneer's criterion of the separation of the lateral parapodial lobes from the ventral sole would seem a much less important character. In any event, this distinction is largely a matter of the particular height on the lateral surface at which the parapodia are given off. While creeping members of both suborders (like *Philine* and *Aplysia*) are equipped with a distinct sole, this character is surely an adaptive one, and it is likewise not surprising to find swimming members of both the Bullomorpha (*Gastropteron*) and the Aplysiomorpha (*Akera*) which have a very similar reduction of the creeping surface and enlargement of the parapodia. The parallel between *Gastropteron* and *Akera* is an instructive one, showing as it does the proneness of both these groups of tectibranchs to produce swimming members. Each group has further given rise to a section of the permanently swimming Pteropoda, the Bullomorpha producing the Thecosomata, and the Aplysiomorpha probably the Gymnosomata.

Two 'bullomorph' characters are generally relied on by those who would include *Akera* in the Cephalaspidea, namely the well-developed shell containing the mantle cavity with its pallial caecum, and the nervous system with its long visceral commissure still retaining the primitive torsion. Such features are each primitive ones: their possession implies not so much a close resemblance to the bullomorphs as a primitive position near the point where the aplysiomorphs sprang from basal tectibranchs. The pallial caecum is an elaborate structure following—independently of the visceral mass—a spiral course around the upper whorls of the shell. The earliest description was by Perrier & Fischer (1911), and Fretter & Graham (1954) have recently given a detailed account of its structure and functioning in the primitive bullomorph, *Actaeon tornatilis*. This caecum was previously regarded as a bullomorph specialization, and its presence in an aplysioid is admittedly not easy to explain. Yet many difficulties disappear if we regard it not as a peculiarly bullomorph character, but as an adaptation likely to be found generally in those tectibranchs with a large mantle cavity, where a sedimented or silty habitat raises problems of pallial sanitation.

Guiart (1901) gave a good description and figure of the nervous system of *Akera bullata*, and it is a pity that Pelseneer's later work (1906) entirely omitted mention of it. In reporting its streptoneurous condition, Guiart compares the nervous system of *Akera* with that of *Actaeon*. Undoubtedly *Akera* is much more primitive in the nervous system than any other aplysioid. It stands at the base of that group with much the same relationship to later Aplysiomorpha as *Actaeon* bears to later bullomorphs. Guiart finds in *Akera* certain characters of the nervous system in which this form already foreshadows the later aplysiomorphs. 'Il nous suffit de considérer un instant le système nerveux de l'Acera pour constater qu'il présente avec le système nerveux de Bulléens un certain nombre de modifications qui vont aller en s'accentuant chez les autres Aplysiens.'

The remainder of the family Akeratidae as recognized by Thiele cannot—it would seem—be placed with *Akera* in the Aplysiomorpha. Mr T. J. Evans (personal communication) informs one of us that *Cylindrobulla* and *Volvatella* are in fact saccoglossans. The different fates of the two subfamilies of Akeratidae is a good example of how—with better knowledge—the basal parts of more advanced groups may be found to encroach into the primitive group of Bullomorpha.

We are indebted to Mr M. Little of Plymouth for bringing our attention to the presence of *Akera* in the Devonport reservoir. A film of the swimming of *Akera* was taken by Mr F. A. J. Armstrong, upon which the diagrams illustrating the swimming movements (Text-fig. 1) have been based. Dr D. P. Wilson kindly supplied the photographs reproduced in Pl. I.

SUMMARY

The tectibranch mollusc *Akera bullata* is recorded for the first time in the Plymouth area, where it was taken in April 1954 in a sea-water tank at Devonport. Large numbers of specimens of all sizes were present, and copulation and egg-laying were in progress. A summary is given of the records of previous occurrences in S.W. England, with ecological notes on the behaviour of the animal at Plymouth. The characteristic swimming movements of *Akera* occur mainly in the spring, and may be correlated with the breeding season. An account of the external form and structure is given and the mode of swimming is described in detail. The digestive system was investigated from Plymouth material, and the structure and mode of action of the alimentary canal described and shown to be typically aplysioid. The remaining characters of the animal, such as the structure of the reproductive organs, the pallial glands and the nervous system, also show aplysioid resemblances, and it is concluded that the family Akeratidae (in part) must be removed from the Bullomorpha and located more correctly in the Aplysiomorpha.

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EXPLANATION OF PLATE I

Swimming movements of *Akera*. 1, 2, lateral view of swimming specimens, slightly less than natural size. 3, a specimen, viewed from above, about to rise from the bottom of a dish. About twice natural size.

Photographs from Kodachrome originals by D. P. Wilson.

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THE FUNCTIONAL MORPHOLOGY OF *OTINA OTIS*, A PRIMITIVE MARINE PULMONATE

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(Text-figs. 1–12)

The family Otinidae is the smallest and probably the least known among the pulmonates. Thiele (1931) places it at the base of the Basommatophora, the more primitive order of the subclass Pulmonata, in the Stirps Actophila. It contains a single species, *Otina otis* Turton, with a geographical range confined to the coasts of the British Isles and north-west France. Its northern distribution reaches as far as the Firth of Clyde, according to Jeffreys (1869), and over the greater part of its British range it appears to follow fairly closely the distribution of the barnacle *Chthamalus stellatus*, which here reaches its northern limit. *Otina otis* is a tiny snail, and its external form is limpet-like. The shell, which is well described by Jeffreys (1869), and also by Ellis (1926), measures up to 2·5 mm in length, with a short apical visceral coil at the posterior end. It is dark chestnut brown in colour, and most resembles a minute *Haliotis*.

A description of the ecology of *Otina otis* has already appeared, as portion of a study of the crevice-dwelling animals of the upper intertidal zone at Wembury (Morton, 1954). *Otina* is rather restricted in its mode of occurrence as regards both vertical tidal range and selection of substratum. It lives a short distance within the mouths of crevices between layers of foliaceous rocks, such as Dartmouth Slate at Wembury and Whitsands, and felsite at Kingsands, and in fissures between blocks of Staddon Grit at Rum Bay and volcanic rocks at Drake's Island. Its vertical range at Wembury extends from EHWN tide to the splash zone of MHWN. A favourite site is upon the fringe of *Chthamalus stellatus*, extending just within a crevice upon a shaded north-facing side, or upon an encrusting layer of the brown alga *Ralfsia*, with the serpulid *Spirorbis borealis* somewhat deeper in the crevice. In conditions of high humidity *Otina* ventures farther out upon the exposed barnacle fringe, where its favourite habit is to hide in the empty shells of *Chthamalus*, together with specimens of *Littorina neritoides*, *L. rudis* and *Lasaea rubra*. The ecological relation between *Otina* and *Chthamalus* would appear to be very close; and the geographical distribution and the distribution of the two species upon the shore to a large extent coincide.

An essentially similar choice of habitat seems to be shown by *Otina otis* where it occurs on the coast of north-west France. The Inventory of the

Roscoff Fauna (Station biologique de Roscoff, 1951) records it under stones, in crevices between the rocks, and in empty balanoid shells. It is further stated to shelter in little tufts of algae, as it does sometimes at Plymouth in clumps of the lichen *Pygmaea pumila* (= *Lichina pygmaea*), and in the byssus of mussels in the middle to upper tidal zone.

METHODS

By virtue of its compact shape and small size for sectioning *Otina otis* is a relatively easy mollusc to study anatomically. During the present investigation animals were dissected alive and after fixation with aqueous Bouin's fluid and with the Dubosq modification of Bouin's. The animals survive for only a short time in the still water of a laboratory vessel and are intolerant of more than a few hours' continued submergence. Living animals were removed from the shell under the higher power of the binocular, and placed in sea water in a cavity slide, where they could be readily dissected with needles, the separate organs of the visceral mass dissociating easily and remaining intact for observation of ciliary and muscular activity in the living state. Dissected preparations were transferred to the monocular for more detailed observation with the ⅔ objective. Fixation in Bouin's for 24 h was found sufficient to dissolve the shell entirely, after which it was found advisable to remove with needles the brown-pigmented shell membrane, to assist quick penetration of the clearing and embedding media and the cutting of clean sections. Specimens were examined as cleared transparent objects, both unstained and after staining lightly with Mayer's haemalum, and were embedded for sectioning in paraffin at m.p. 52–56° C. Transverse and longitudinal sections were cut at 5 and 8 μ thickness. Staining with Heidenhain's azan was found to give ideal histological results, especially for details of the inclusions in the cytoplasm of gland cells and for general nuclear staining. For finer details of ciliary apparatus, recourse was made to iron haematoxylin without counterstaining. Mucicarmine and thionin were each used to detect mucus, and Masson's trichrome, as recommended by Pantin (1946), was found a useful alternative general stain to azan.

EXTERNAL FEATURES

The animal in life, as it creeps about in moist air, is not completely covered by the shell, which is raised above the substratum to expose the sides of the fleshy foot. These—like the rest of the body—are translucent white in colour; in the crawling animal they are usually kept tumid with blood. The head bears a pair of large rounded lappets, which extend forward to overlap the mouth in front, and almost reach the substratum. Behind them, the buccal mass is rounded and bulbous, appearing pinkish, with a narrow black jaw, visible through the white integument. The cephalic tentacles are short and blunt, springing from the head behind the lappets. They appear flattened and triangular from above, and the eyes are located at the centre of their bases. The sole of the foot is oval in outline. It is divided by a deep transverse groove, which cuts off the semicircular anterior third of the foot from the longer posterior portion. The animal progresses on a firm surface by advancing the front of the foot over the substratum, and fixing it in a forward position while the rest of the foot is brought up behind. The whole sole is ciliated and glandular. The most elaborate gland of the foot, however, is the suprapedal gland (Fig. 2, *spg*) which opens on the upper or anterior surface of the front lobe. The suprapedal gland forms a compact cluster of mucus-secreting cells, embedded in the connective tissue of the anterior lobe of the foot. Their contents stain grey-green with Masson's stain, bright pink with mucicarmine. Their ducts interpenetrate between the cells of the overlying columnar epithelium, and a forward ciliary current carries a sheet of secretion to the anterior edge of the foot in the neighbourhood of the mouth. The epithelium overlying the gland is raised in an oval pad of ciliated and gland cells, which may form a cushion or a shallow trough according to the state of contraction of the foot. Farther backwards, towards the base of the foot, the subepithelial gland cells spread upwards to enclose the sides of the epithelial tract, which leads back into a recess, lined with squamous cells. Here a dense cluster of cell ducts thrusts its way to the surface, from the posterior part of the gland, where the cytoplasm differs in being less granular, the secretion staining pale green with Masson's. The whole of the secretion of the suprapedal gland appears to find its way forward to the point of contact of the mouth with the substratum. It plays the chief part in compacting particles of food into a bolus in which they are raked up by the odontophore and drawn into the mouth, with a further secretion of mucus from the buccal glands. Within the gut, the salivary glands are small, and the oesophagus almost non-glandular. The supply of mucus from the foot thus provides the chief binding substance added to the food before it is conveyed to the stomach.

The intrinsic muscles of the foot are arranged in two extensive bands inserted on the shell dorso-laterally, just inside the rim of the aperture. From either side, bundles of fibres radiate downwards into the foot, and spread out to be inserted over the whole width of the sole. They serve, on contraction, to draw the shell close to the ground, and, with the blood contained in the pedal sinus, to bring about the general movements of the foot itself. The suprapedal gland lies anteriorly in a triangular space above the convergence of the two muscle bands. Above the level of the gland, and behind it the foot is crossed by a strong sheet of transverse muscles, which form a long, shallow basin, flooring the haemocoele and containing the viscera above.

The pallial opening (Fig. 1, *p op*) is set rather far back on the right side, close to the posterior end of the foot. The anus opens immediately in front of it. The mantle forms a wide skirt, fitting closely over the head and trunk, and reflected as a thin white rim at the edge of the shell. The roof of the mantle cavity is a thin-walled, triangular area, quite transparent and with no special respiratory capillaries. It is longest on the right side, where the kidney (Fig. 2, *kd*) lies postero-dorsally to the pallial opening. The kidney is a small, pyramidal sac, with smooth, unfolded lining, its broad base applied behind to the anterior lobe of the digestive gland. The renal pore opens into the pallial cavity just inside the pallial aperture. Farther back, the pallial cavity extends as a wide, shallow recess, lying to the right of the mid-line and not shown in Fig. 2. It is overhung by the anterior lobe of the digestive gland, which penetrates into the mantle. The pallial opening, or pneumostome, forms a short, narrow tube lined by a thick sleeve of gland cells (Fig. 4). On the lateral wall, the epithelium is shorter than on the mesial, and is continuous with the pallial skirt below its insertion on the trunk.

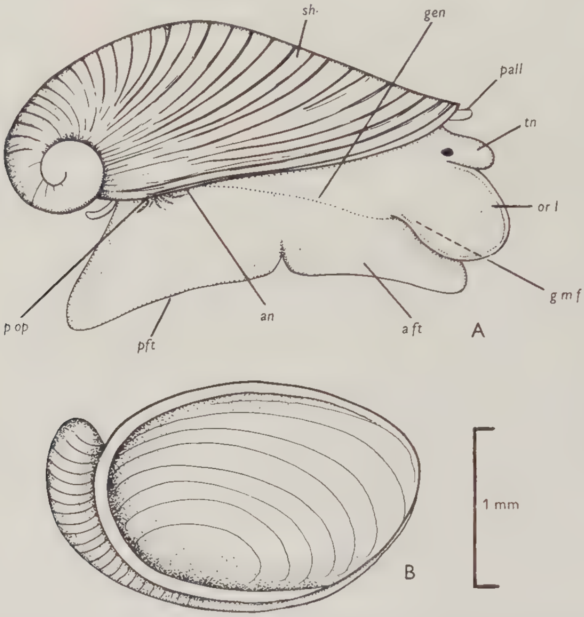

Fig. 1. *Otina otis.* A: lateral view of the entire animal. *aft*, anterior lobe of the foot; *an*, position of the anus beneath the pallial skirt; *gmf*, position of the male and female genital apertures beneath the right oral lappet at its posterior edge; *gen*, course of vagina and vas deferens beneath the integument; *or l*, oral lappet; *pall*, projecting margin of the mantle; *pft*, posterior lobe of the foot; *pop*, opening of pallial cavity; *sh*, shell; *tn*, cephalic tentacle. B: the shell, viewed from beneath.

The pallial opening in limpet-like gastropods (Graham, personal communication) appears to be always a profusely glandular region. A number of different mucoid secretions are produced, which probably have both a lubricatory and a protective role. In *Otina* there appear to be three distinct types of subepithelial gland cell (Fig. 4). The first and second types are much the greatest in length (150 μ) and penetrate deeply against the haemocoele on the mesial side. In the first (Fig. 4, *gl* 1) the cytoplasm forms a coarse reticulum, staining lightly brown in azan; the cell contents are often removed during fixation. The second type of gland (*gl* 2) stains bright blue in azan. The third type of gland (*gl* 3) is cylindrical or flask-shaped and is a good deal shorter. The cytoplasm stains various shades of red after azan, towards maturity the cell contents appearing deep wine red to purplish or dark blue. Intermixed with these cells, just beneath the surface of the epithelium, are often smaller, rounded cells, uniformly packed with brown staining spherules, and probably representing younger stages of the first type of gland. Towards the pallial cavity the epithelium of the pneumostome flattens to a squamous form and glands of the third type are especially numerous. At the extreme inner edge of the gland mass, there is often a nest of several large-nucleated, spherical cells (Fig. 4, *replc*), which evidently serve as replacement cells for discharged glands.

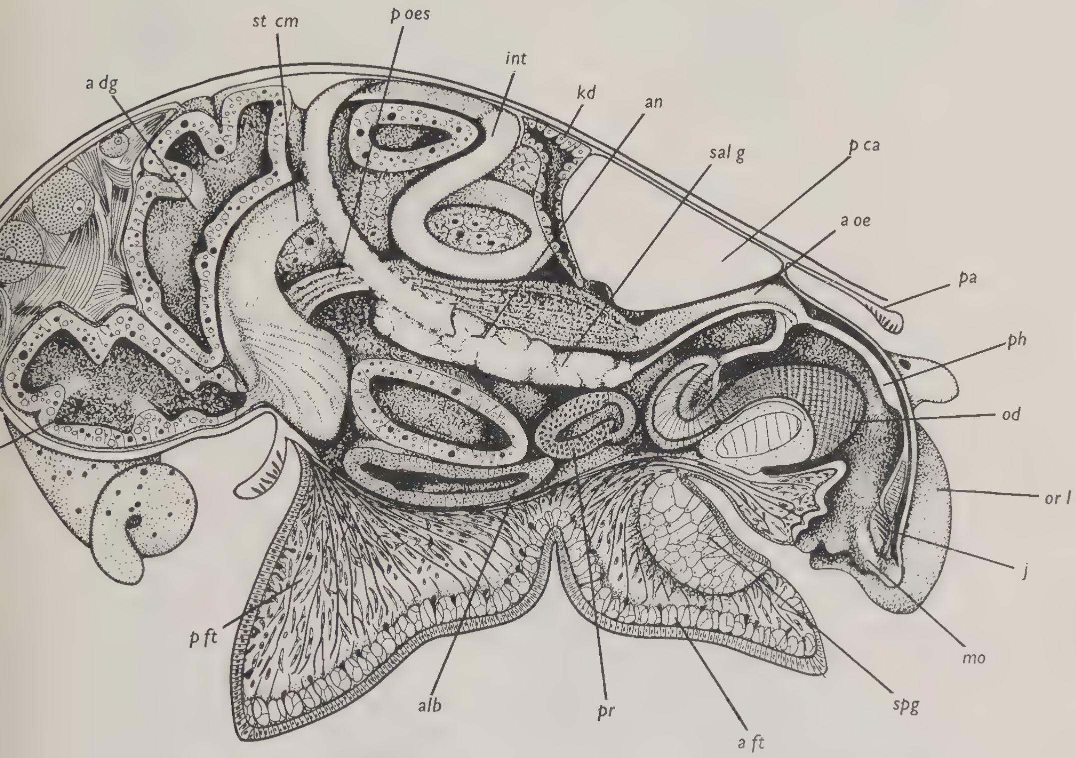

Fig. 2. *Otina otis.* Semi-diagrammatic longitudinal view of the entire left half of the animal, sectioned along the sagittal plane. The course of the rectum, from the mid-line to the anus, on the right side, has been indicated by broken lines, although actually lying outside the sagittal plane. *adg*, anterior lobe of the digestive gland; *aft*, anterior portion of the foot; *alb*, tubule of the albumen gland; *an*, position of the anus to right of sagittal plane; *aoe*, anterior region of the oesophagus; *gon*, ovotestis; *int*, intestine; *j*, jaw; *kd*, kidney; *mo*, mouth; *od*, odontophore; *or l*, oral lappet; *pa*, mantle; *p ca*, pallial cavity; *p dg*, posterior lobe of the digestive gland; *pft*, posterior division of the foot; *ph*, buccal mass; *poes*, posterior region of the oesophagus; *pr*, prostate; *salg*, salivary gland; *spg*, suprapedal gland; *st cm*, 'style sac'.

The sole of the foot (Fig. 3 B) is lined with columnar epithelium of uniform height (15 μ) with a central row of ovoid nuclei, and a short but dense ciliary

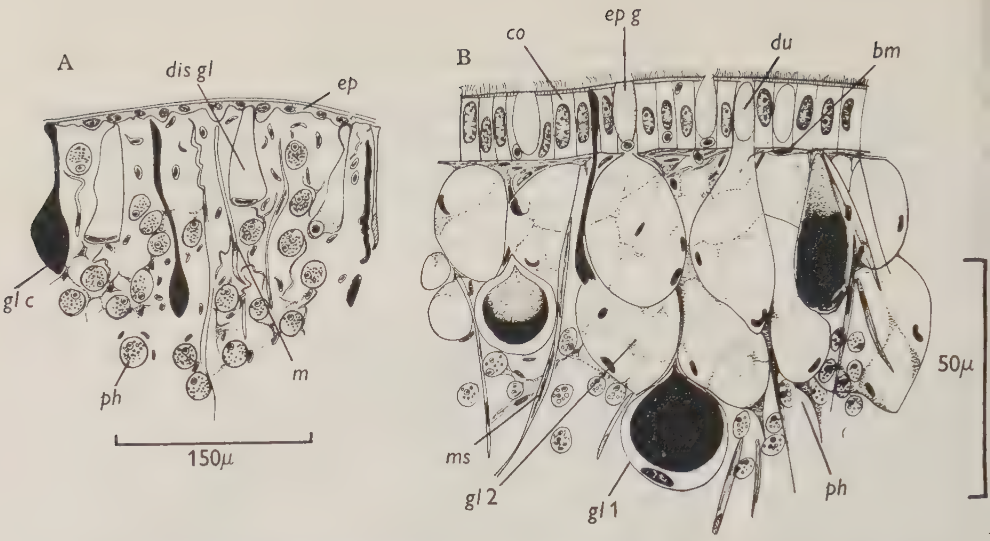

Fig. 3. *Otina otis.* A: transverse section of the integument in the lateral region of the foot (from preparations stained with Masson's and with azan). *disgl*, discharged gland cell; *ep*, squamous external epithelium; *glc*, gland cell; *m*, muscle strand; *ph*, phagocytes in blood sinus. B: transverse section through the epithelium of the sole of the foot (Bouin's; Masson's trichrome). *bm*, basement membrane; *co*, columnar epithelial cell; *du*, duct of a subepithelial gland cell; *epg*, epithelial gland; *gl* 1, *gl* 2, types of subepithelial glands; *ms*, muscle fibre; *ph*, phagocyte.

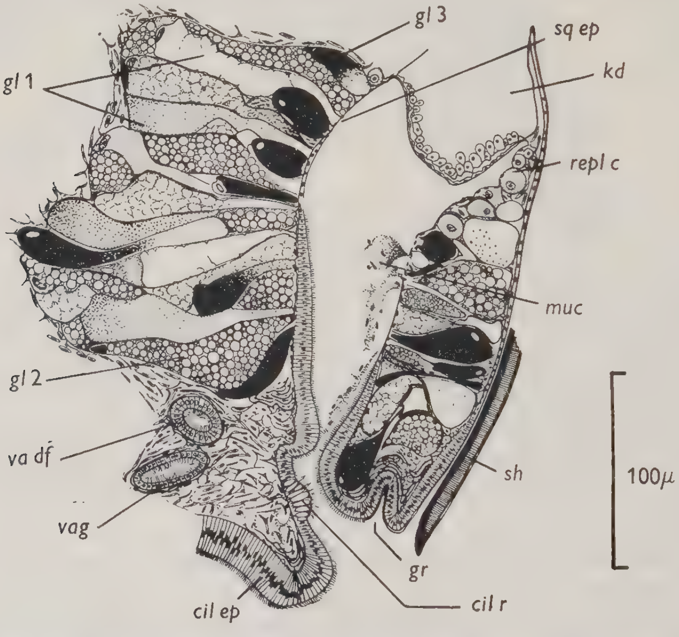

Fig. 4. *Otina otis.* Transverse section of the pallial cavity near its opening, showing the histology of the epithelium lining the pneumostome (from preparations with Masson's trichrome, and azan). *cilep*, ciliated columnar epithelium; *cilr*, ciliated ridge running along the side of the foot below the closure of the mantle cavity; *gl* 1, *gl* 2, *gl* 3, types of gland cells of the subepithelial layer as referred to in the text; *gr*, secretory groove along the pallial margin; *kd*, kidney; *muc*, mucus discharged into the pallial cavity; *replc*, replacement cells; *sh*, shell; *sqep*, squamous epithelium; *vadf*, vas deferens; *vag*, vagina.

coat. Between the ciliated cells appear the ducts of large subepithelial glands. Occasionally a whole gland cell, complete with basal nucleus, lies entirely within the columnar layer. Amoebocytes are usually numerous, wandering in from the subepithelial layers. The basement membrane of the epithelium is rather muscular, and incorporates a dense sheet of collagenous connective tissue, to which are attached narrow slips of muscle from the deeper layers of the foot. There are two types of glands, clustered thickly together in supporting connective tissue. The first (*gl* 2) are ovoid and rounded, much the more numerous and crowded in an almost continuous sheet beneath the basement membrane. They stain bluish in azan, light green after Masson's and in haematoxylin pale purple. Thionin leaves them dark brown. The nuclei are basal or parietal and the ducts are stout and cylindrical, penetrating between the overlying columnar cells. The second type of gland (*gl* 1), much less numerous, stains deeply purple or black in haematoxylin, deep red with azan. It is commonly elongate or fusiform, with a short stout duct running up through the epithelium. Sometimes one or more of these glands becomes depressed beneath the lighter staining cells, and its cell body then appears perfectly spherical with a long dark-staining duct thrusting its way into the epithelium.

The sides of the foot, which in the living animal are exposed to the moist air, are quite different in histology from the sole (Fig. 3 A). Their subepithelial connective tissue forms a large blood sinus, lying on either side laterally to the muscles of the foot, from which it is penetrated by narrow strands of muscle inserted against the epithelium. The external layer is thin and squamous, not ciliated, and with a very narrow refractile border. The basement membrane is rather crenulated by the bulging against it of cell nuclei. Glands are of a single type, staining very darkly in haematoxylin and deep red in azan. These cells form long (100–150 μ) vesicles of secretion running deeply into the connective tissue, usually with a flattened nucleus lying horizontally at the cell base. What their function may be is not ascertained. They are probably similar in nature to the dark staining glands (*gl* 1) of the sole, and may have a protective or antiseptic function, similar to that of the multicellular glands described by Fretter (1943, p. 697) in the integument of *Onchidella*. Within the blood spaces of the sides of the foot, amoebocytes are very numerous (*ph*), rounded or slightly ovoid after fixing, with a small round, uninucleolate nucleus. They are filled with tiny granular inclusions, staining reddish brown in Masson's, as well as large amorphous clusters of particles. It seems likely that the broad lateral tracts of the foot may in *Otina* carry on accessory respiratory and excretory activity, especially in view of the reduced size of the pallial cavity, its lack of a respiratory plexus, and the reduction in size of the kidney. At the sides of the foot, blood is brought to the external surface of the body, enclosed only by the thinnest of epithelia through which respiratory interchange might easily be possible with the surrounding air.

An increase of respiratory surface by epithelial folds is probably unnecessary, in view of the small volume of the animal and its comparative lack of active movements. The amoebocytic cells probably have an excretory role, following on reduction of the kidney and the lack of a pallial water current to carry discharged renal products out of the mantle cavity. A similar occurrence of excretion through the integument by amoebocytic action has been described in several other marine gastropods, for example *Omalogyra* (Fretter, 1948) and the *Pyramidellidae* (Fretter & Graham, 1949). Here the laden amoebocytes emerge along a thin strip of epithelium into the pallial cavity. In *Osilinus lineatus*, Nisbet (personal communication) has observed the excretion of injected particles of carmine by the amoebocytes of the foot.

ALIMENTARY CANAL

The Buccal Region

Otina feeds like a limpet by the rasping action of the radula, browsing over the rock surface, and picking up wave-lodged diatoms and detritus. The places where it lives are often not rich in debris; the food tends to be more finely selected than in ellobiids (see Morton, 1955 *a*), and the radula is kept constantly at work raking particles into the buccal cavity. The alimentary canal is not much specialized, and presents many features primitive among pulmonates and reminiscent of microphagous prosobranchs. The mouth is a long ventral slit between the oral lappets. The suprapedal mucous gland opens just below it, between the bases of the lappets, and its secretion passes forwards, helping to consolidate a food bolus as the radula works against the substratum. The buccal bulb is relatively large, and occupies the whole of the haemocoele of the convex head. When the animal is extended, the head projects forward from beneath the mantle skirt. The bulb is strongly muscular, especially in front of the odontophore and around the mouth. Its cavity leads dorsally from the mouth; when the muscles forming the oral sphincter are relaxed, the bluntly pointed odontophore is thrust down and the radular ribbon unfolded and licked across the substratum.

Fig. 5 A shows a schematic view of the buccal bulb, opened from above. In front of and below the tip of the odontophore, the buccal cavity is laterally compressed; it forms a vertical cleft between two smooth, cuticle-covered side walls. The cuticle is penetrated by the ducts of mucous cells clustered beneath the epithelium, and forming on either side a lateral buccal gland, secreting binding mucus as the food is taken in. The secretion is in part released at the level of the substratum, as the radula is actually at work. The buccal glands stain pale blue with azan, red with mucicarmine and black after thionin. Just within the mouth, the anterior wall of the buccal cavity is reinforced by a small crescentic jaw, running across the expanded dorsal channel between the glandular side walls. It serves, when the mouth is opened, to grip on the substratum, providing purchase during the strokes of the radula. It consists

of a series of close-set, spinulose rods of chitin, embedded in a pad of thickened cuticle. Along the base of the jaw, runs a transverse plate of 'cartilage', consisting of long vacuolated cells, invaded by trabeculae from the surrounding connective tissue.

The odontophore (see Fig. 5 A) is bluntly pointed in front, and covered over its whole free surface by the wide radular membrane, which bears small

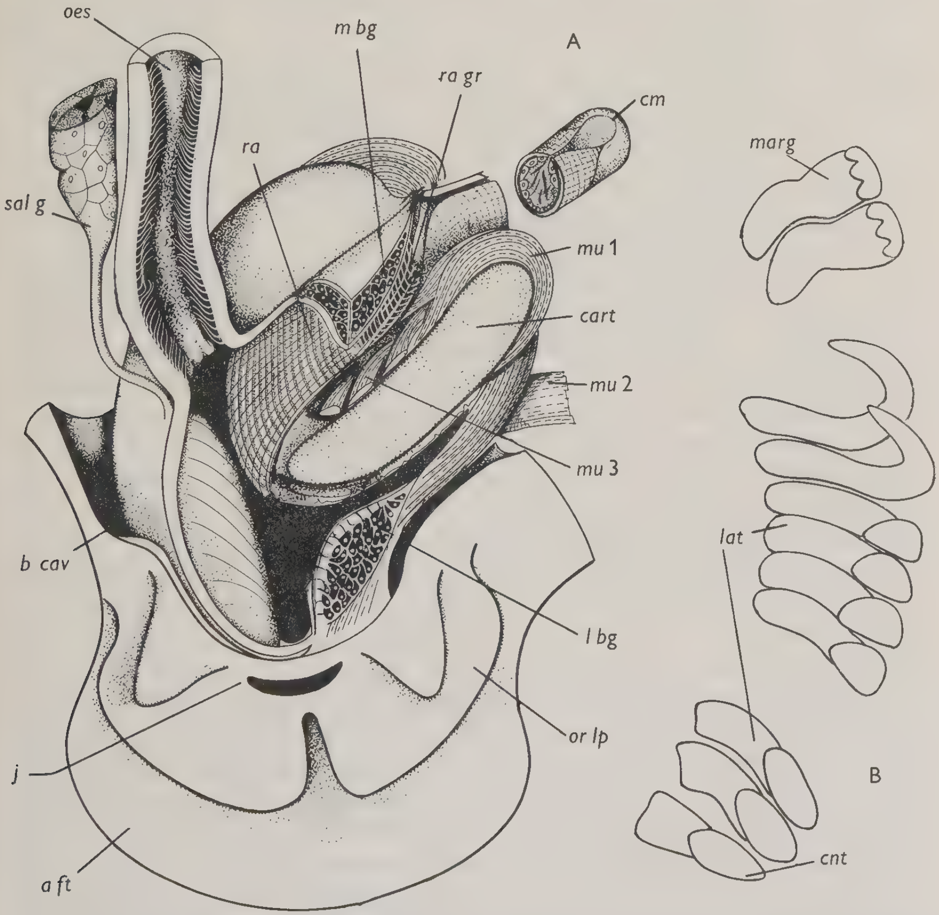

Fig. 5. *Otina otis*. A: Stereogram illustrating the structure of the buccal mass. The haemocoele of the head has been laid open from in front and the left half of the oesophagus and the roof of the buccal mass removed. The radula is represented as being cut away on the left side to show the odontophoral cartilage and the arrangement of the chief muscles of the buccal mass. *aft*, anterior division of the foot; *bcav*, haemocoele of the head; *cart*, odontophoral cartilage; *cm*, radular caecum; *j*, jaw; *lbg*, lateral buccal gland; *mbg*, median buccal gland; *mu* 1, *mu* 2, *mu* 3, muscles of the buccal bulb referred to in the text; *oes*, oesophagus; *orlp*, oral lappet; *ra*, radula; *ragr*, radular groove; *salg*, right salivary gland. B: representative teeth of the radula. *cnt*, central tooth; *lat*, lateral teeth; *marg*, marginals.

uniform teeth. The radular structure is illustrated in Fig. 5 B. Each row consists of a central tooth, flanked by a series of up to 100 laterals, with a further series of marginals towards the edges. The central tooth forms a narrow wedge, widest along its flat posterior edge, and surmounted by a single elongate cusp. The laterals are rather long and arcuate, resembling in shape the teeth of the single lateral pair of the mesogastropod radula. They have single, bluntly pointed cusps. The marginals are similar in shape, equipped with three short blunt cusps of roughly equal size. The odontophore is supported by a single horseshoe-shaped cartilage, with the two side lobes laterally compressed and giving insertion to muscles operating the radula. The substance of the cartilage is traversed by muscle fibres, which serve to compress and elongate it as the radula is protracted. The cytoplasm of the cartilage cells is permeated with fine black pigment granules, which impart a dense greyish colour to the whole cartilage.

The radular ribbon curves widely round the free edge of the odontophore, and narrows posteriorly, its edges becoming upcurved to form a shallow trough open from above. Running backwards into the radular caecum, the margins converge towards the dorsal mid-line, so that the radular membrane is rolled into an incomplete tube, extending around the lining of the caecum, attached to its epithelium. The radular caecum (Fig. 5 A, *cm*) is a short tube, 80–90 μ in diameter, passing backwards between the side limbs of the odontophoral cartilage, and emerging behind as a rounded bulb containing the tooth-secreting cells, or cuspidoblasts. At the opening of the caecum from the buccal cavity, its lumen is entered anteriorly, and along the dorsal fissure between the edges of the radula, by a long column of connective tissue; called by Carriker (1946), in *Lymnaea*, the 'collostyle'. This forms a central core filling the whole of the radular caecum; its rounded tip projects bluntly from the caecum into the buccal cavity.

The buccal cavity above the odontophore is wide and dorso-ventrally depressed; it reaches backwards behind the oesophagus and is floored by the radula which extends downwards at the sides of the odontophore. It is roofed in the middle line by a tapering flange of ciliated epithelium triangular in section, which fits closely from above into the trough of the radula, and dwindles towards the mouth of the radular caecum. Through the epithelium open the ducts of subepithelial mucous cells, similar to those of the lateral buccal glands. They may be referred to as the median buccal gland (Fig. 5 A, *mbg*), and their secretion probably serves chiefly to pick up particles borne backwards beyond the oesophagus.

Fig. 5 A shows also the principal muscles of the buccal bulb. These comprise extrinsic retractor muscles, passing forward from the foot and the floor of the haemocoele to the base of the odontophore and the side walls of the buccal cavity. There is an especially large pair of radular retractors originating on the outer posterior aspects of the odontophoral cartilages, passing inwards

beneath the cartilages, and running forwards underneath the radular caecum, on which they are inserted ventrally. Other muscles, with insertions on the inner aspects of the cartilage pass obliquely forwards, above the cartilage, to a broad insertion beneath the radular ribbon. They contract to fold the ribbon into a trough and to draw its edges together.

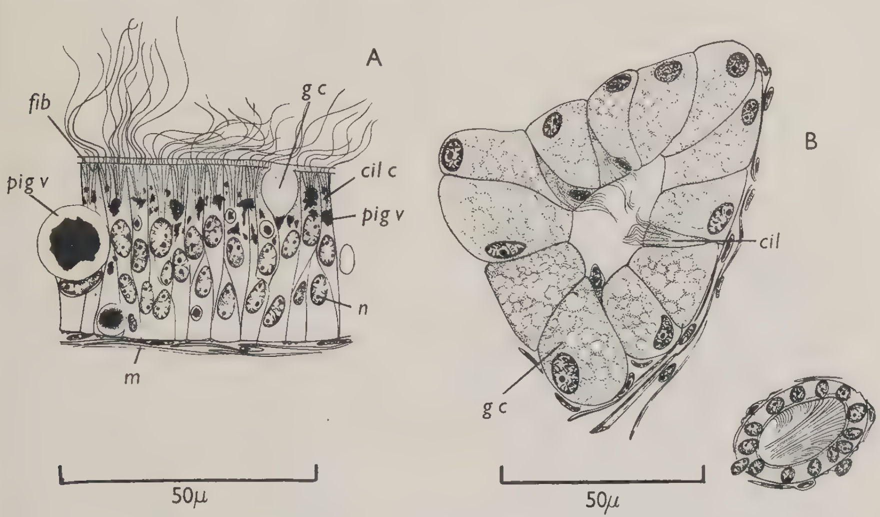

Fig. 6. *Otina otis*. A: epithelium of the anterior region of the oesophagus (Bouin's; azan) *cil c*, ciliated cell; *fib*, intracellular fibrillae; *gc*, glandular cell; *m*, muscle fibre; *n*, nucleus of ciliated cell; *pig v*, vacuole containing black pigment. B: Transverse section of a salivary gland, and of its duct (Bouin's; azan). *cil*, ciliated cell; *gc*, gland cell.

The Oesophagus

The oesophagus (Fig. 5 A, *oes*) arises rather far forwards from the buccal bulb in front of the odontophore, immediately above the mouth. The odontophore when retracted lies entirely behind the oesophageal opening. The oesophagus funnels upwards from the buccal bulb, and runs back in the mid-line as a narrow cylindrical or dorso-ventrally flattened tube, 100–120 μ in diameter. This region may be referred to as the anterior oesophagus. Immediately behind the buccal mass it terminates abruptly, and the gut widens into a thinner-walled fusiform crop, which may, at times, be strongly distended with a mass of food material.

Along either side of the anterior oesophagus, extending backwards beside the crop, lies a long salivary gland. This consists of a thick-walled tube, opaque white or slightly translucent, and tapers in front to a very fine duct which penetrates the pharyngeal roof. The structure of the salivary gland (Fig. 6 B) is extremely simple: it is 90–100 μ across, cylindrical or sometimes prism-shaped by compression from adjacent structures. The lumen is surrounded by up to a dozen rows of gland cells, of a single type with rather coarsely reticulate cytoplasm, staining light blue in azan, grey-green in Masson's, jet black in thionin, and pink in mucicarmine. The cell contents are thus entirely mucoid in nature. The nuclei are rounded or ovoid, basal or pressed close to the side wall of the cell, and are uninucleolate. Two or three small ciliated cells usually appear in a transverse section, wedge-shaped and single, staining darker blue in azan, and with little tufts of lash-like cilia, short (8–10 μ) and usually inconspicuous. The salivary duct is 35–40 μ across, with small cuboidal cells, which are not glandular. They bear a coat of tall cilia extending to the centre of the lumen, and lashing the mucoid secretion of the gland forward to the buccal cavity.

The lining epithelium of the anterior oesophagus (Fig. 6 A) is of a uniform height throughout, never thrown into folds, and is backed by a narrow layer of longitudinal muscle. The cells are tall columnar (40 μ), with very long cilia, keeping up a lashing beat backwards and sweeping and rolling small mucous food boluses into the crop. The cilia are 25–30 μ long and extend half way across the narrow lumen. The superficial portion of the cytoplasm stains rather more densely than the rest, and there is a narrow striated layer at the cell surface, through which the cilia penetrate, with a series of fine, perpendicular intracellular fibrillae extending downwards to the central row of nuclei. The nuclei are broadly ovoid, light-staining and binucleolate. Gland cells are rather rare in the anterior oesophagus, forming small, plump bodies or fusiform cells, light blue in azan, about a quarter or one-third the length of the ciliated cells and inserted between their distal tips. Granules of black pigment are incorporated in the cytoplasm to the superficial side of the nuclei; pigment may also be aggregated in rounded spherules, some of them quite large, within amoebocytic cells, which frequently wander into the epithelium from the underlying layers. When the crop is empty its wall is thrown into small, impermanent folds. Like the anterior oesophagus, the wall has a densely speckled appearance, from the presence of black pigment incorporated in the epithelial cells. The crop is marked off sharply from the anterior oesophagus, by its much greater diameter (300–400 μ) and by the reduction in size of the epithelial cells, which are short (12–15 μ) with cilia of about the same length. The nuclei form a single basal row, and gland cells are infrequent, as in the anterior oesophagus. Pigment is scattered in small granules in the superficial part of each cell.

The Stomach and Intestine

The stomach (Fig. 7 A) is a triangular sac, lying on the left side of the animal at the base of the visceral mass. Its left aspect is visible through the body wall, tapering in front into the proximal limb of the intestine, which runs straight forward just beneath the thin external integument. The oesophagus enters towards the mid-line on the deep aspect. For the most part, the stomach is thin-walled and transparent. Its contents are clearly visible through the wall, and it often becomes greatly distended when filled with recently ingested food. Towards the apex on the left side, the wall becomes rather more muscular, to form a short, pointed pouch (Fig. 7 A, *mp*). There are two digestive diverticula, the lobules of which make up the greater part of the

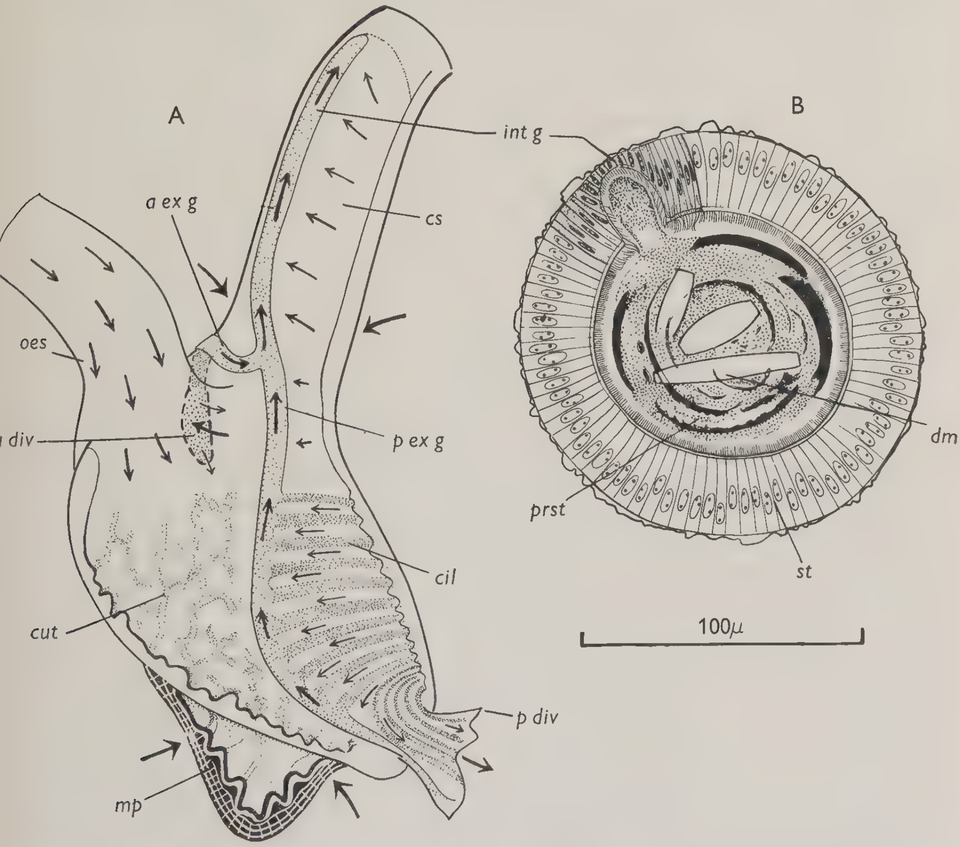

Fig. 7. *Otina otis*. A: the stomach, figured without dissection as a transparent object, showing the course of the ciliary currents. The heavy black arrows indicate the movement of contents into the diverticula, and (outside the stomach) the regions of the strongest muscular contraction. *adiv*, anterior digestive diverticulum; *aexg*, anterior excurrent groove; *cil*, ciliated area; *cs*, 'style sac'; *cut*, area of cuticle; *intg*, intestinal groove; *mp*, muscular pocket; *oes*, oesophagus; *pdiv*, posterior digestive diverticulum; *pex g*, posterior excurrent groove. B: transverse section of the proximal limb of the intestine, or 'style sac'. *dm*, diatom enclosed in the mucus of the protostyle; *intg*, intestinal groove; *prst*, protostyle; *st*, epithelium of the style sac.

visceral mass. They are few in number, and each is large and rounded, or bluntly lobulate, orange or dark brown in colour, with large black spherules speckling the surface. The larger anterior digestive diverticulum opens through the antero-dorsal wall of the stomach. Its aperture is situated close to the entry of the oesophagus and some distance behind the origin of the intestine. The somewhat smaller posterior (morphologically left) diverticulum opens at the postero-median angle of the stomach, close to the mid-line. From each diverticulum, the individual lobules run together to form a single cavernose pouch; the glandular walls are pocketed into rounded saccules, and the lumen of each pouch is much more spacious than the stomach itself.

The internal structure of the stomach is very straightforward. The left and antero-dorsal wall is covered with thin cuticle (Fig. 7 A, *cut*) thrown into tiny, irregular wrinkles, which become obliterated in the full stomach. Posteriorly, in the more muscular pocket, the cuticle is thrown into several stronger, semi-permanent folds, formed by differences in the height of cells. The muscle coat is formed here by a stronger layer of circular fibres, closely intermixed with longitudinal. The posterior pocket of the stomach serves as a 'gizzard' of the simplest form, but is never strongly demarcated from the rest of the stomach as in other Basommatophora. Its muscle coat merges into the thinner contractile wall of the anterior part of the stomach. The right median half of the stomach is ciliated (Fig. 7, *cil*) provided with ten to twelve transverse plicae which sweep round towards a ciliated groove (*p ex g*) running up the median edge of the stomach, and leading from the posterior digestive diverticulum to the intestine. It is joined by a short tributary from the anterior diverticulum, and continues into the proximal part of the intestine as a longitudinal intestinal groove. The diverticula open into the stomach by narrow slits. The mouth of the posterior diverticulum is somewhat pocketed outwards from the stomach and its walls folded, and lined with fine cilia, which beat towards the stomach. Throughout each diverticulum the epithelium is uniformly glandular. Ciliated cells hardly extend outwards beyond the stomach. The stomach wall is entirely devoid of glands; the only secretory region is a rim of cells, often well-marked, at other times hardly apparent in sections, around the mouth of the anterior, or sometimes both diverticula. They are non-mucoid, staining bright red in azan. They produce a viscid substance which appears to have the function of binding the particles from the digestive gland as they are carried across the ciliated lips of the diverticula into the stomach.

The strongest cilia within the stomach are developed in the excurrent grooves from the diverticula. Here a rapid current sweeps smaller particles forward into the intestine. The grooves do not themselves clear the whole of the waste material from the digestive gland; large clusters of spherules from the digestive cells frequently appear in the general contents of the stomach. The ciliated ridges are formed by variations in the height of the cells of the columnar epithelium, which reaches 30 μ along the folds, but less than half this length along the furrows. There is a median row of long rod-like nuclei, tending to be more compressed in the narrower cells which constitute the folds. The cilia are short (2–3 μ) and very dense. Though individual particles may be swept along the furrows, the ciliated area as a whole is evidently not adapted for sorting. The primary function of its cilia is not to deal with separate particles, but to assist the rotation of the heavy bolus of stomach contents bound with mucus. The cuticle-bearing cells are stouter than the ciliated cells, and are 30 μ tall, with a subcentral row of oval nuclei. The cuticle layer is thin (1–2 μ), staining lightly blue with azan and is attached by a delicate layer of fine tag-like processes resembling cilia.

The proximal limb of the intestine leading forward from the stomach is very distinct in histology from the stomach and from the rest of the intestine. Its lining epithelium is of brick-shaped columnar cells (Fig. 7B), 30 μ in height, with a central row of clear-staining, ovoid nuclei, each with two nucleoli. The cytoplasm is uniformly granular and rather lightly staining. Mucous glands are never developed. The distal half of each cell is occupied by a fan of intracellular fibrillae connected with a row of short bristle-like cilia, 5–6 μ in height. The lining of the intestinal groove is, by contrast, of extremely narrow cells, of which there are a dozen or so on either side of the groove, with compressed nuclei, darker staining cytoplasm. The cilia are longer and finer, keeping up a rapid beat aborally along the intestine. The bristle-like cilia of the rest of the epithelium beat in a transverse direction. Their action is difficult to see in the excised intestine in which they are generally inert. The direction of the effector stroke is, however, often retained in the row of cilia after fixation, and this fact, together with the concentric arrangement of layers of mucus and faeces, indicates that these cilia serve to rotate the faecal rod, which fills this part of the intestine and projects into the stomach. In short, both in its structure and function, the proximal part of the intestine in *Otina* possesses all the features of a style-sac of the most elementary kind.

It has become clear, from the work of Graham (1949) (see also Morton, 1952*a*) that the style-sac form of stomach is a primitive and generalized feature in the earliest members of the molluscan phylum. The most archaic members of both the gastropods (Archaeogastropoda and Cyclophoracea) and the lamellibranchs (Protobranchia) have strong cilia in the proximal part of the intestine, serving to rotate a 'protostyle', or rod of mucus and faeces projecting into the stomach. The protostyle provides the chief source of movement within the stomach, both for drawing in a food-string from the oesophagus, and for sweeping particles over the ciliary sorting area. In later evolved types in both classes, the rod may lose its faecal character and become clear and hyaline, to form the enzyme-containing crystalline style. In *Otina* waste particles from the stomach and 'faeces' from the digestive gland are compacted into a protostyle which is slowly rotated by cilia; particles are circulated within the stomach. The protostyle is much more coherent than the rest of the contents of the stomach, and while it rotates, it is gradually passed backwards into the intestine by the cilia of the longitudinal groove, and forms the first portion of the faecal string. The mucus of the protostyle is deposited in thin layers, made up from two types of secretion, the bluish staining (azan) secretion carried into the stomach from the anterior part of the gut, and the bright red-staining secretion from the cells lining the rim of the digestive diverticula.

The mode of action of the stomach of *Otina*, a primitive pulmonate, is thus very similar to that of the lamellibranchs *Nucula* (Graham, 1949) and *Malletia* (Yonge, 1939), and of the prosobranch *Murdochia* (Morton, 1952*b*). As in the protobranchs, muscular action also plays an important part in the working of the stomach. Contractions of the wall, especially in the stouter posterior pocket, serve in part to triturate the contents of the food bolus, which is pressed firmly against the cuticularized wall. The cuticle forms a protection against abrasion by sharper fragments, and corresponds in location to the gastric shield. At the same time, regular contractions of the whole stomach wall serve to force semi-fluid material squeezed out of the food bolus (with the finest particles in suspension) into the digestive diverticula, which are, in *Otina*, filled chiefly by muscular action. From time to time, especially after an excretory phase of the digestive gland, the opening of the intestine shows more pronounced muscular contractions, and strong peristaltic waves nip off portions of the protostyle, which are carried backwards rapidly and added to the faecal string. Though the cilia of the intestinal groove are still active, it appears to be chiefly by these sudden muscular contractions that the faeces are driven into the intestine. In general, the food ingested by *Otina* comprises particles of a finer grade than in *Leucophytia*. Diatoms bulk large among the stomach contents, and sufficient trituration is evidently afforded by the muscular wall of the stomach, in the absence of a highly differentiated gizzard as in the Ellobiidae.

The stomach of *Otina*, in its structure and mode of action, appears to be the most primitive yet described in a pulmonate. It shows almost diagrammatically most of the landmarks regarded by Graham (1949) as typical of the stomach in the earliest molluscs: thus, for example, its division into ciliated and cuticularized halves (though there is no spur-like gastric shield), the presence of a 'style sac' with a protostyle, and the paired digestive diverticula with excurrent grooves converging to form an intestinal groove. The posterior gastric caecum (held by Graham to be an archaic feature) is not developed at all in *Otina*, unless we regard it as represented by the outpocketing of the stomach from which the posterior digestive diverticulum opens. This caecum is however present in the more primitive ellobiids (Morton, 1955*b*); the earliest bivalves, the protobranchs (Yonge, 1939) and the Archaeogastropoda (Graham, 1949) have it also. If a posterior caecum is to be regarded as a feature of the primitive molluscan stomach, the structural simplification going with reduction in size has probably been responsible for its loss in *Otina*.

Further, however, the stomach of *Otina* clearly foreshadows the condition in the higher Basommatophora. Already it has to a large extent lost its reliance on cilia, and muscular action has increased in importance. At the same time, *Otina* serves to explain what has hitherto not been clear, the relation of the muscular pulmonate stomach to the style-sac type of stomach in prosobranchs, where the chief reliance is on ciliary systems and mucus secretion.

The '*style-sac*' in *Otina* passes without change in diameter, into the middle region of the intestine which forms a narrow tube of uniform width (75 μ). The limb running forward from the stomach passes upwards through the anterior lobe of the digestive gland, to emerge at the dorsal surface. Here it forms a short loop, commencing along the left side, then turning sharply backwards to encircle the anterior lobe of the digestive gland behind, and running forward on the right side, to open by the anus, just in front of the pallial opening. It is lined with four to five longitudinal ridges formed by differences in cell height. The cilia beat strongly out of the grooves, and towards the anus along the summits of the ridges. The epithelial cells are columnar, 12–15 μ in height, the ciliary coat 5 μ tall. Mucous glands are rather few, plump and fusiform in shape, and contain small clusters of secretion droplets staining bright red in azan. The subepithelial layer is only sparsely muscular, but the faecal string is moulded by intermittent muscular action, and carried backward by peristaltic waves, as well as by the continuous fast beating of the cilia. The ridges which line the intestine are temporarily flattened as the string is passed along. The rectum differs from the middle intestine in its usual lack of folds, its shorter cells (10 μ) and longer cilia (7–8 μ). The gland cells are of the same kind but are a good deal more numerous, filled with separate secretory spherules and constricting the narrow ciliated cells. Their secretion provides the final mucous covering of the long rope of faeces. The muscle coat is rather better developed than in the middle intestine, and the whole, or at least long portions, of the faecal string may be voided by peristaltic waves at one time. As compared with the prosobranchs, the faecal string in *Otina* is only loosely compacted. It is never compressed into separate pellets, and, by reason of the external position of the anus, there arises no problem of the fouling of the pallial cavity by voided faeces.

The Digestive Gland

The digestive diverticula in *Otina* are the site of both absorption and enzyme production; they have also an excretory role. The lining epithelium, from the stomach to the tips of the tubules, is glandular, and contains cells of two chief types, digestive cells in greater numbers, interspersed with smaller groups of excretory cells. At the absorptive phase of the digestive gland (illustrated in Fig. 8A), the cells are for the most part short and columnar, of a uniform height 35 μ, with rounded or suboval nuclei lying slightly above the basement membrane. The largest of the excretory cells are broad-based

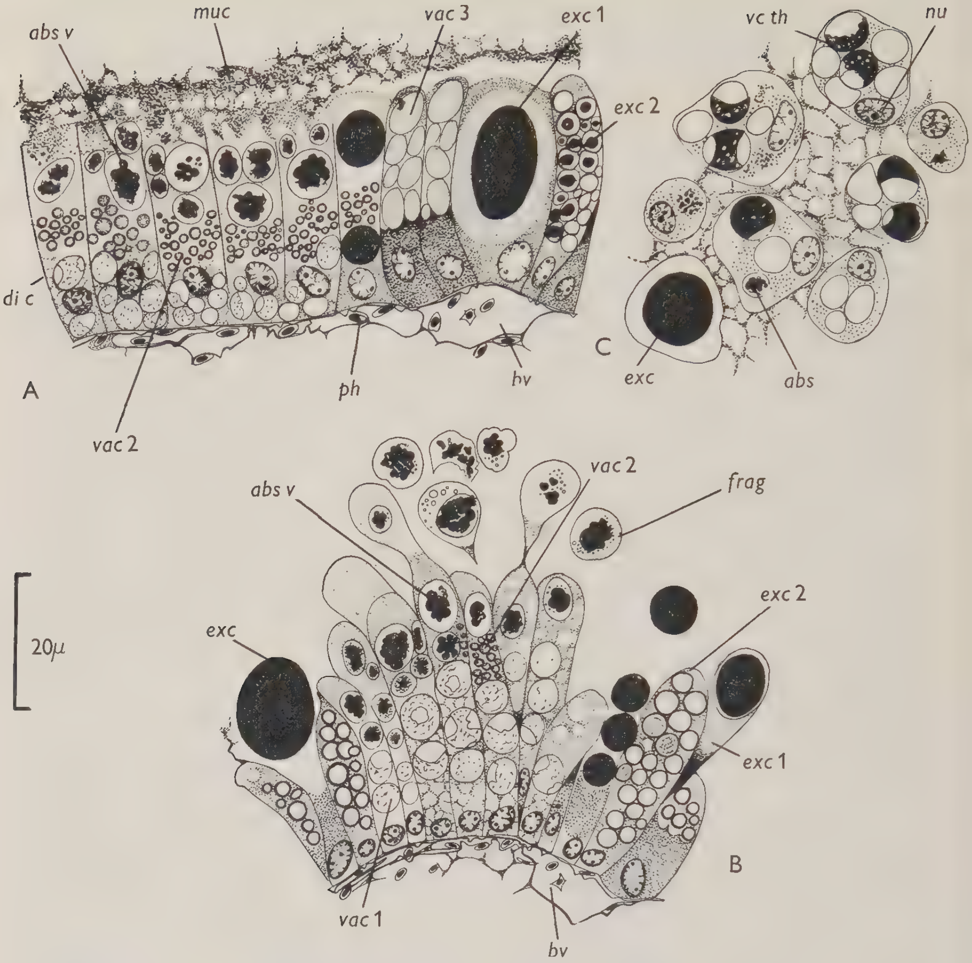

Fig. 8. *Otina otis*. A: epithelium of the digestive gland, at the phase of absorption. B: the same at the phase of fragmentation and excretion (Bouin's; azan). *absv*, food vacuoles loaded with absorbed material from the lumen; *bv*, blood vessel; *dic*, digestive cell; *exc*, excretory spherule extruded from a cell; *exc* 1, excretory cell of the first type; *exc* 2, excretory cell of the second type; *frag*, fragmented tip of digestive cell constricted off into the lumen; *muc*, mucus from the stomach, with finely divided food material in suspension; *ph*, phagocytic blood cell; *vac* 1, basal vacuole of the digestive cell; *vac* 2, smaller excretory (?) vacuoles in the digestive cells; *vac* 3, blue-staining (azan) vacuoles in the excretory cells at the absorbing phase. C: 'fragmentation phagocytes' formed by abstriction of nucleated portions of the digestive cells, suspended in mucus within the stomach. *abs*, granules of absorbed material from stomach; *exc*, excretory spherule; *nu*, nucleus of 'phagocyte'; *vcth*, basal vacuole of digestive cell (after thionin staining) with stained surface 'membane'.

and triangular, and as a rule may be distinguished from the digestive cells by the larger size of the nuclei (5–6 μ).

The digestive cell (Fig. 8, *dic*) contains several types of inclusion; its free border is flat and entire at the ingesting phase, and the cytoplasm near the surface is filled to a depth of 4–5 μ with the mucoid material containing suspended food particles, with which the lumen of the tubule is distended. This material is being freely taken up along the whole surface of the digestive epithelium, so that the cell border is often obscured, the cytoplasm taking on the same coloration (blue in azan, green in Masson's) as the contents of the lumen. Below the ingesting region, the cytoplasm is filled with large spherical vacuoles, 6–7 μ across, as a rule two or three deep in each cell, a single vacuole filling the whole cell width. Many of these vacuoles contain droplets of ingested material. They are in some places small and no more than 1–2 μ across, elsewhere clumped together in irregular clusters, or coalescing into a spherule filling the whole vacuole. The rest of the vacuoles remain colourless with most stains, including azan, haemalum, Masson's and mucicarmine. They stain strongly, however, with thionin, which could be employed to distinguish them from all other cell inclusions of the digestive gland. Their appearance after thionin is shown in Fig. 8 C; the vacuole consists of a colourless and structureless central sphere, surrounded by a thin membrane which in fixed material almost always ruptures and peels away at one or more points. With thionin the membrane becomes deep indigo blue; with other stains it presents merely an uneven refractile surface, colourless or pale golden-yellow. At the base of the digestive cell, clustered around and sometimes below the nucleus, is another zone of these thionin-staining vacuoles, of rather smaller diameter (*vac* 1), perfectly colourless, without trace of ingested contents. They sometimes extend into the middle of the cell but usually, above the nucleus, are to be found inclusion spherules of a third type, of smaller size (2–3 μ) pinkish brown in living macerations and retaining the same colour in azan or haemalum. These are evidently excretory in character, though it is not certain whether they are extracted from the blood or represent the final indigestible residue of material absorbed from the lumen.

Excretory cells appear, at the absorptive phase of the digestive gland (Fig. 8, *exc*) to be represented by three stages. The largest are pyriform, strongly expanded towards the tip, and each containing a large, black or dark brown, excretory sperule 15–20 μ in diameter. In each cluster of excretory cells appear one or more columnar, round-tipped cells, filled with twenty to thirty light brown or pink spherules, contained in separate, clear-staining vacuoles. These spherules may sometimes be discharged separately in the lumen, although, as a rule, the columnar cell appears to be an earlier stage of the pyriform cell, in which a single large spherule is later formed by the coalescence of the smaller ones. The remaining excretory cells are also columnar during the ingesting phase, the basal half of the cytoplasm being pinkish in azan, or violet with thionin. The distal half is filled with rounded or ovoid vacuoles, 5–6μ across, which do not stain with either thionin or azan.

At the excreting phase (Fig. 8 B) the epithelium of the digestive gland takes on a rather different appearance. The digestive cells become longer and round-tipped. They bulge strongly into the lumen and their free tips now begin to be constricted off, forming spherical fragments of cytoplasm (*frag*) loaded with blue-staining (azan) contents. This material would seem to represent an indigestible residue of the mucus-borne particles that were previously absorbed into the digestive vacuoles. The smaller pink spherules appear to be shed also by fragmentation or rupture of the cell wall, while the remaining basal part of the cell is occupied by the large thionin-staining vacuoles. The larger excretory cells containing the densely black spherules have now become greatly distended, some with a row of two or three spherules, others with a single large one. These are shed into the lumen by rupture or by fragmentation of the cell. The smaller columnar excretory cells are either packed entirely with purplish brown spherules; or their basal halves may be of uniformly staining cytoplasm, pink in azan, violet in thionin, while the distal end develops separate pinkish spherules, as in the excretory cells at the earlier phase (Fig. 8 A).

In *Otina* the spherical fragments of digestive cells, cast off during the excretory phase, appear for the most part to be rejected particles laden with waste material. On reaching the stomach, they become plastered on to the protostyle or pass directly into the intestine. In some individuals of *Otina*, however, sectioned after 2 days' starvation, the digestive epithelium presented a ragged, highly fragmented appearance. The stomach was filled with a watery mucus, containing, together with excretory spherules, large nucleated portions, roughly spherical, or of rather irregular shape, derived from the bases of the digestive cells. The nuclei were generally polar in position, and the cytoplasm was vacuolated and filled with clear-staining spherules, giving the thionin reaction, like those of the basal parts of the digestive epithelium (Fig. 8 C). These fragments of the digestive gland are of interest in closely resembling the 'phagocytic cells' described by Millott (1937) in the nudibranch *Jorunna*, as originating by abstriction from digestive cells. Forrest (1951) has also observed them in a number of other dorids. They differ from the fragments of the digestive gland extruded into the stomach in prosobranchs, and the constricted tips of the digestive cells in *Otina*, in retaining nuclei, and, according to Millott, presumably some power of free movement. In *Jorunna* they absorb, and in part digest, such particles as introduced blood corpuscles within the stomach, providing, as it were, a means of non-localized intracellular digestion. In *Otina*, from the small size of the stomach and the difficulty of introducing experimental foods, no evidence could be gained as to their role in digestion. Their thionin-staining vacuoles may be presumed, as in the digestive cells, to be the source of enzymes.

From the following experiments, it is clear that the stomach in *Otina* contains active enzymes.

The substrate employed was the gelatin film of a developed photographic plate. Whole digestive glands, and separate stomachs from which all traces of digestive glands had been carefully removed, were pressed out on the plate and kept moist under cover-slips. The stomachs selected were empty of food or detritus, and filled with watery mucus containing yellowish brown cell fragments from the digestive gland. After 24 h, the gelatin film beneath the crushed stomachs had been in each case completely and cleanly digested away. Digestion by the stomach fluid proceeded in fact much more rapidly than by the macerated digestive glands themselves, where the gelatin became much softened but only slightly eroded from the plate. Obviously, in *Otina*, digestion is by no means all intracellular, and material absorbed by the digestive cells has evidently undergone previous breakdown by enzymes in the stomach. Enzymes are active in the stomach after a day's starvation, and it would appear that their origin is to be sought in the so-called 'fragmentation phagocytes' derived from the digestive cells.

Wandering amoebocytic cells from the subepithelial blood cells appear to play little or no part in digestion in *Otina*, either in the stomach or in the digestive gland. In the wall of the stomach they are extremely scarce or absent. This is in contrast to their frequent presence in prosobranchs (*Struthiolaria* (Morton, 1951)); *Lunella* (Turbinidae) (Morton, unpublished) and in lamellibranchs (Yonge, 1926). They appear to be characteristic of stomachs with wide ciliary sorting surfaces, and often wander through the epithelium of the sorting area in very large numbers. Their comparative absence in *Otina* and in opisthobranchs may be correlated with the reduction in importance of the sorting area. Ciliary sorting and amoebocytic ingestion appear as a rule to go hand in hand.

Reproductive System

Like the Ellobiidae dealt with in a previous paper, *O. otis* is a protandrous hermaphrodite. The genital system is of the most primitive type found among the pulmonates; the duct is undivided and hermaphroditic throughout, except for a bifurcation into male and female channels at the extreme anterior end, in front of the glandular genital mass, and only a short distance behind the external openings. As in *Leucophytia*, the gonad shows a marked occurrence of two phases, a sperm-producing phase occupying the autumn and earlier winter, followed by an egg-producing phase culminating in oviposition. The condition of protandry is most evident from the condition of the gonad itself; the glandular genital tract reaches its female condition in advance of the completion of spermatogenesis, and at no time during sexual maturity are the female ducts absent. The prostate, however, always displays its greatest development during the male phase, and is reduced during the female phase, when the development of the albumen gland and mucous gland is accentuated. For the sexual development of *Otina otis* over a single reproductive season, a cyclic diagram may be constructed which differs in scarcely any feature from that presented for *Leucophytia* (Morton, 1955*a*).

The arrangement of the accessory glands is illustrated in Fig. 9. The little hermaphrodite duct conveys sex products from the gonad, to open into the large or glandular hermaphrodite duct. The albumen gland opens into the large hermaphrodite duct at the same point. The proximal part of the large hermaphrodite duct forms the posterior mucous gland. As it runs farther forward, the duct retains its single lumen, though it is divisible on histological grounds along its whole length into an anterior mucous gland, constituting the female tract, and a prostate, forming the male channel. A non-glandular conducting tube, the distal common duct, takes its origin from the region of the anterior mucous gland and prostate, and continues forward along the floor of the haemocoele, towards the right side of the head. It divides anteriorly into a narrow vas deferens and vagina. From the vagina, just in front of this bifurcation, arises a large bursa copulatrix. A second sperm storage sac, the accessory bursa copulatrix opens by a long stalk from the distal common duct, immediately behind the origin of the vagina and vas deferens. Pelseneer's short description and figure (1901) is thus fairly accurate in outline, though the albumen gland and posterior mucous gland are indicated only diagrammatically, and the relations of the anterior part of the tract are not shown in detail. No previous account exists of the histology or functions of the genital tract of *Otina*.

The hermaphrodite gland or ovotestis forms a single pouch lying on the concave surface of the visceral spire, applied to the posterior lobe of the digestive gland. Its cavity is partly divided by thin trabeculae of connective tissue bearing the germinal cells and giving to the gonad externally the appearance of a cluster of rounded follicles. The first part of the little hermaphrodite duct is a narrow transparent tube, which courses forward ventrally, close to the mid-line, and before reaching the albumen gland becomes strongly convoluted, its coils often being distended and sacculated (*lhd*). These coils are opaque white through the greater part of the year, containing large amounts of stored sperm, and thus function as a simple vesicula seminalis. The epithelium is of simple columnar or cubical cells with round nuclei and with a long ciliary coat. There are no glands, and only a very sparse muscle coat, two or three fibres in thickness, both longitudinal and circular. Resorption of sperm was never observed, nor do the sperms become orientated.

The structure of the large hermaphrodite duct may be followed from Fig. 9. The little hermaphrodite duct opens into it at the base of the posterior mucous gland (*p muc*) which forms a rounded translucent sac, lying to the right side of the oesophagus, about half way along the trunk, beneath the thin pallial floor. The albumen gland (*alb g*) is applied to the posterior mucous gland

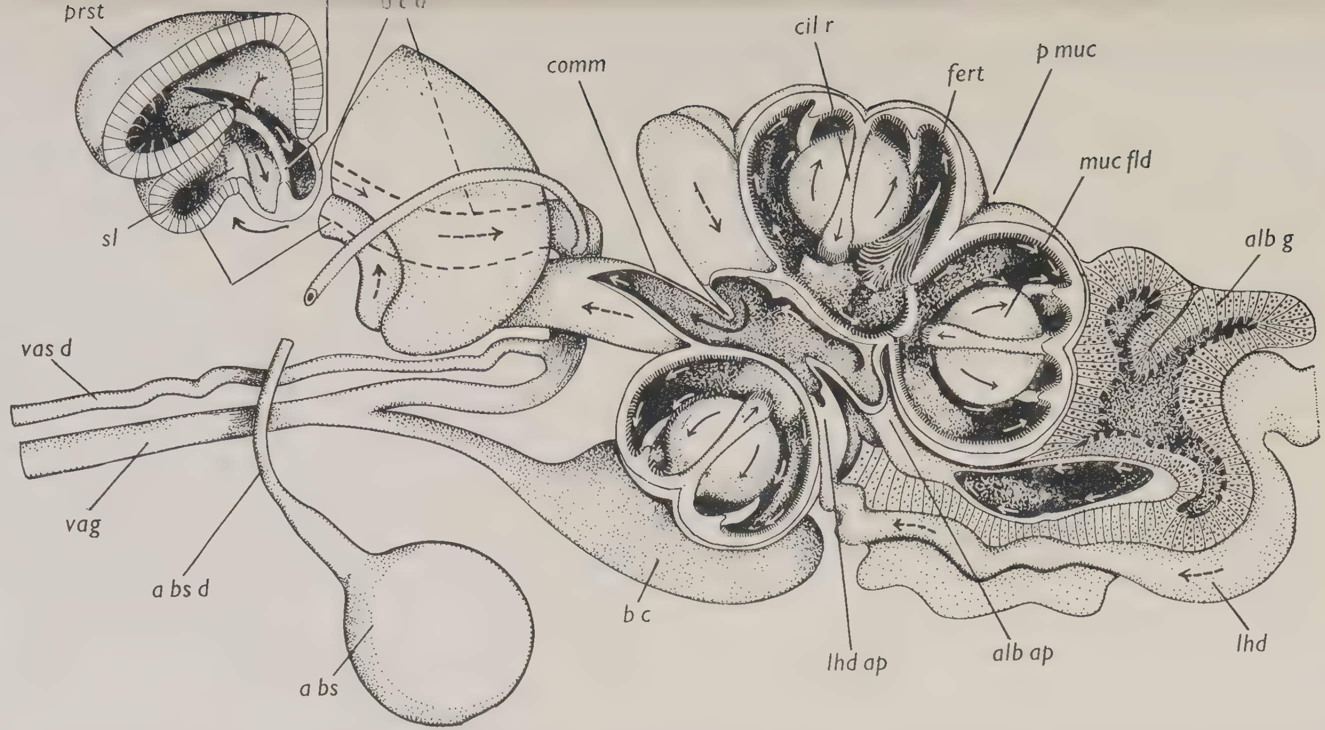

Fig. 9. *Otina otis*. Stereogram of the glandular regions of the genital tract, viewed from the median aspect and dissected to show the internal structure and the course of the ciliary currents. The anterior-most part of the albumen gland is represented in longitudinal section; the roof of the posterior mucous gland has been removed; and the prostate with the anterior mucous gland and distal common duct has been cut across transversely and the two halves separated to show the relations of the three ducts. The anterior parts of the vagina and vas deferens, with the penis, have been omitted. *abs*, accessory bursa copulatrix; *absd*, duct of the accessory bursa copulatrix; *albap*, opening of the albumen gland into the posterior mucous gland; *albg*, albumen gland; *comm*, communication between the anterior and posterior mucous glands; *bc*, bursa copulatrix; *cdfld*, longitudinal fold dividing the cavity of the distal common duct; *cilr*, ciliated ridge running round a fold of the posterior mucous gland; *dcd*, distal common duct; *fert*, fertilization pouch; *lhd*, little hermaphrodite duct; *lhdap*, opening of the little hermaphrodite duct into the posterior mucous gland; *mucfld*, glandular fold of the posterior mucous gland; *pmuc*, posterior mucous gland; *prst*, prostate; *sl*, longitudinal slit by which the prostate and anterior mucous gland communicate; *vag*, vagina; *vasd*, vas deferens.

behind, opening directly through its ventral wall by a short wide duct, immediately in front of the little hermaphrodite duct.

The albumen gland in *Otina* forms essentially a series of long diverticula discharging into the conducting channel of the genital tract; its cavity is no longer, as in the prosobranchs, traversed by eggs or sperm. It is built up of a series of opaque, yellowish white lobules, which are irregularly fused together anteriorly, towards the base of the gland. The longest lobule forms a thick strap passing backwards near the ventral mid-line. The smaller lobules are blunt and finger-shaped, each penetrated by a small cleft opening into a wider lumen at the base of the gland. Towards the egg-laying period, the lobules become very stout, packed with white droplets of secretion, tightly filling the haemocoele, around the anterior lobe of the digestive gland.

The histology of the albumen gland (Fig. 10A) is of the simplest type; the lining is formed of tall epithelial gland cells, and there are no subepithelial glands as in higher pulmonates and most opisthobranchs. There are two types of cell, smaller and shorter wedge-shaped cells, and much more numerous columnar gland cells. Both bear cilia, which are, however, lost in the gland cells shortly before the time of secretion. The gland cells are 40–50 μ in length and have a uniform width of 9–10 μ. The basal nuclei are almost spherical, darkly staining, with a single prominent nucleolus. The albumen spherules range in size from large ovoid droplets (6 μ) to smaller rounded granules scattered densely through the cytoplasm. They stain bright red with azan, red with Masson's, pale pink with haematoxylin, and remain colourless after mucus stains. The wedge-shaped cells are generally short and triangular, inserted between the free tips of the gland cells. They are sometimes longer and attenuated, half the length of the glands. Their nuclei are spherical or compressed according to the situation of the cell. The cilia form long tufts, 8–10 μ tall; the lumen is fairly continuously ciliated, except at the time of secretion, when the ciliated cells tend to become dislodged and squeezed out into the lumen, during the rupture of the gland cells.

The posterior mucous gland is rather complex in structure. It is built up of three plump globular pouches, arranged in a compact rosette, so that the whole gland is of trefoliate shape in horizontal section. Each pouch is in turn subdivided by a rounded fold of glandular epithelium (Fig. 9, *muc fld*) the three folds extending radially towards the centre of the gland, alternately with the pouch walls. The whole lumen is thus divided into six radial clefts, communicating with each other at the centre. The entrances from the little hermaphrodite duct (*lhd ap*) and the albumen gland (*alb ap*) lie ventrally, at the middle of the rosette, and here also the anterior part of the glandular hermaphrodite duct (consisting of the anterior mucous gland and the prostate) makes its exit. The roof of each of the three pouches forms a horseshoe-shaped tract of thickened, glandular epithelium, and each horseshoe is indented by a tongue-shaped radial groove which marks the base of the internal fold. The outer wall of each pouch is of ciliated and glandular epithelium, 15–20 μ in height. The dividing fold is formed by a septum of connective tissue with sparse muscle fibres. On either side of the septum arises a hemispherical group of gland cells (25 μ) with rounded, basal nuclei. Their contents are colourless or pale blue in azan, bright red in mucicarmine and black with thionin (Fig. 10B). Interspersed with the gland cells are ciliated cells, narrow and tapered, but somewhat shorter than the glands. Their nuclei are compressed and rod-like and the cells broaden at the free surface to bear a tuft of short cilia. The mucous cells become very plump during their secretory phase, but are at other times much less developed, and the glandular fold is only slightly thicker than the rest of the lining of the pouch. Around the mid-line of the fold runs a narrow ridge (Fig. 9, *cilr*) made up wholly of ciliated cells. These form a wedge-shaped cluster in section, fanning out from the edge of the septum of connective tissue. The ciliary currents in the mucous gland are shown in Fig. 9. Over each hemisphere of gland cells, currents beat downwards towards the base of the fold, while along the bottom of each fissure runs a narrow ridge (*ri*) along which currents beat inwards towards the centre of the lumen. Along the ciliated tract *cilr*, running round each fold, the ciliary beat is also radial, towards the middle of the mucous gland.

It appears that the eggs pass through each of the three pouches of the posterior mucous gland in succession, receiving thin mucoid capsules before being carried forward into the anterior mucous gland. At the centre of the posterior mucous gland, where the little hermaphrodite duct opens, the paths followed by the eggs and sperm become separated. Sperm is directed forward into the anterior mucous gland-prostate portion of the common duct, and bypasses the mucus-secreting pouches. From the arrangement of the pouches, it would appear that the eggs first pass from the central lumen into the fertilization pocket (Fig. 9, *fert*) which is a wide dorso-ventral slit, formed simply as an outgrowth of the lateral pouch of the mucous gland. It is lined with columnar epithelium, 30 μ tall, which contains no glands, and is distinguished by its very long cilia (100–120 μ), extending in a flame-like tuft through the whole length of the slit. The fertilization pocket is directly continuous at its entrance with the wide duct of the albumen gland; its columnar cells merge into the shorter ciliated epithelium, which lines the albumen duct and isolated cells with albumen granules sometimes occur amongst the cells at the base of the ciliary flame. The cilia of the fertilization pocket could not be induced to beat in excised material; but the function is probably to expel the fertilized eggs, with their albumen coat, into the remaining portion of the mucous gland. The eggs appear to pass next through the two divisions of the posterior pouch, thence through the middle pouch, and arrive finally, surrounded by a mucoid capsule, in the anterior compartment of the lateral pouch. From the anterior wall of this pouch, a wide slit communicates with the anterior mucous gland-prostate division of the genital tract. Sperm as well as ova are conveyed forward along this channel by a common duct which first runs towards the mid-line in a long V, then turns obliquely forwards. The sperm then pass along the prostatic side of the duct, and the eggs along the anterior mucous gland.

The anterior mucous gland (*amuc*) and the prostate (*prst*) lie together on the right side, ventro-laterally to the pharynx, and immediately beneath the thin epithelium of the pallial cavity. The prostate is the more superficial; it is broad and dorso-ventrally compressed, arched against the pallial floor, and is kidney-shaped, convex laterally and concave towards the mid-line. The prostate terminates bluntly at either end; its lumen is spacious, and leads by a wide longitudinal slit, along the concave side, into the anterior mucous gland. The two glands are in open communication for some three-quarters of their common length. The histology of the anterior mucous gland is essentially like that of the posterior. The secreting cells, however, stain deeper blue with azan, and they become distended with mucus at an earlier stage. This portion of the duct is very enlarged and tumid immediately before egg-laying; its cells produce the mucous mass with which the egg capsules are surrounded.

The lining epithelium of the prostate (Fig. 10D) is simple and unfolded, consisting of a single layer of very characteristic secreting cells. These are tall, and stoutly columnar (50×10–$20\,\mu$), and thus a good deal larger than the cells of any other region of the genital tract. Some of the cells are especially wide, and narrowly compress their neighbours. In vertical section they are rectangular or broadly ovoid; the free surfaces are either entire, or broken by the discharge of contents, often bulging convexly into the lumen. The basal nuclei are exceptionally large ($10\,\mu$), almost spherical, though sometimes compressed against the base of the cell. They are densely granular and stain darkly in azan and iron haematoxylin, with two prominent nucleoli. The distal two-thirds of the cell are filled with rounded or irregular secretion granules, averaging 7–8 μ across, closely crowded in the cytoplasm. They stain bright blue in azan, green in Masson's, remaining colourless after haematoxylin or stains for mucus. The prostatic secretion evidently serves as a nutritive component of the semen and can be recognized, persisting with foreign sperm in the bursa, long after copulation. The basal portion of the cytoplasm, in which the nucleus lies, is coarsely but uniformly granular, without separate inclusions, and stains lightly pink in azan. Ciliary cells are rather scarce in the prostate. They form little triangular wedges filled by small compressed nuclei ($5\,\mu$ in length) between the tips of the glandular cells. Their cilia are extremely long, forming whip-like tufts (40–50 μ) trailing into the lumen, and helping to lash the secretion forwards. Beneath the epithelium, the prostate has a rather strong coat of circular muscle, ten to twelve fibres deep, and reaching 5–6 μ across.

The anterior mucous gland and the prostate are accompanied along their whole length, by a third, and much smaller tube, the distal common duct

(Fig. 9, *dcd*). This lies underneath the prostate and immediately lateral to the mucous gland, but is not itself glandular. It is muscular-walled and lined with long cilia, and originates anteriorly from both glands together, towards their blunt, anterior ends. Running at first backwards, the distal common duct soon becomes quite separate from both the mucous gland and the prostate. It conveys both sperms and ova to a point level with the posterior end of the prostate and then turns sharply forwards to run towards the head. The stereogram (Fig. 9) shows the relations of this duct to both the mucous gland and the prostate. Its wall is thrown into three or four impermanent longitudinal folds, and anteriorly, towards the opening from the prostate and mucous gland, its lumen becomes divided by a tall longitudinal fold of the ventral wall; and the duct dilates to about the same diameter as the tapered anterior end of the mucous gland. The prostate and the mucous gland are themselves partly divided by a large fold, which projects obliquely across their common lumen. Anteriorly, the ventral fold of the distal common duct fuses along its free edge with this dividing fold, so as to separate completely the lumen of the prostate from that of the mucous gland, and to form a separate opening from each into the distal common duct. Thus, on the right, a long fissure from the prostate opens into the right compartment of the common duct; into the left compartment opens the mucous gland by a slit-like aperture, which extends forward beneath the prostate, as far as the blind anterior end of the common duct. Farther backwards, the distal common duct narrows, and becomes quite closed off; the dividing fold along its ventral wall disappears. By the division of this duct at the openings from the glandular tract, eggs and sperm are directed separately into the common conducting channel. The subepithelial layers are strongly muscular, and access from either the prostate or the mucous gland can be closed off at need, by approximation of the edges of the slit leading to the distal common duct.

Opposite the posterior end of the prostate, the distal common duct runs down ventrally, and gives off the narrow duct of the accessory bursa copulatrix (Fig. 9, *absd*). It then plunges beneath the sheet of transverse muscle flooring the haemocoele, and runs forward, just beneath the body wall, along the right side of the foot, below the line of fusion of the mantle with the body wall. Almost at once it divides to form two narrow conducting channels, a dorsal vas deferens (*vasd*) and close beneath it, the vagina (*vag*) rather more distensible, but normally of the same diameter as the male duct (30–40 μ). The lining of the vagina varies a good deal according to its state of contraction. As a rule, to within a short distance of the female opening, it is little folded. It is lined with tall columnar cells, with long, rather sparse cilia. There are no gland cells. The muscle coat includes both longitudinal and circular fibres. Just after the departure of the vas deferens, the vagina gives off from its ventral wall a narrow duct, of the same histological structure, with a strongly muscular wall. This duct passes back to enter the haemocoele, and, turning towards the mid-line, expands into an ovoid, dorso-ventrally flattened sac, the bursa copulatrix. The vagina opens by a small, ventrally directed aperture, at the base of the foot, concealed beneath the right oral lappet. Close to the aperture its wall becomes much more distensible than farther back; the epithelium lower and thrown into triangular folds, mainly from differences in the size of the component cells. The long cilia beat outwards, carrying away waste secretions, and probably assisting the muscular contractions of the wall in oviposition.

The short vas deferens has a strong coat of circular muscle. It is lined with tall, ciliated epithelium rather resembling that of the vagina. The cilia are long, reaching the centre of the lumen. The vas deferens runs close to the vagina as far as the female aperture, at the side of which it turns inward to run alongside the penis. The male aperture is a small pore, separate from and just mesial to the female opening. It leads into a short muscular tube, which represents the invaginated penis. The intromittent organ in *Otina* has perhaps the simplest structure yet observed in any pulmonate. The penial tube is perforated at its tip by the vas deferens, and when everted, has a length about equal to that of the vagina as far as the bursal duct. There is no retractile papilla, and the organ forms, in the terminology of Hubendick (1945) a 'pseudopenis'. When invaginated it curves obliquely backward in the body wall, passing mesially to the penial nerve, and to the nerve arising from the cerebral ganglion and going to the oral lappet. Its apex forms a small bulb, lying in the cephalic haemocoele, in contact with the mesial surface of the right cerebral ganglion, close against the roof of the buccal mass. The retractor muscle is a slender strap, running to the left across the pharynx, and inserted on the floor of the trunk, midway along the body cavity.

The penial tube is circular in section, 80 μ in diameter, near the middle of its length. Towards the male opening, its lining is sparsely ciliated, elsewhere thinly cuticularized. Its epithelium forms six triangular longitudinal folds, with underlying muscle fibres. The circular muscle layer forms a prominent external coat. The vas deferens runs to the tip of the penial tube through the connective tissue of one of the folds; it forms here a very narrow tube, 12 μ across, and its dense cilia fill the whole lumen.

Of the two sacs for the storage of sperm, the vaginal bursa copulatrix is the larger (*bsc*), and receives foreign sperm after copulation. It lies closely against the floor of the haemocoele, ventrally to the mucous gland on the right side. The bursa and its duct are both muscular, serving, together with the pressure of blood in the haemocoele, for the expulsion of sperm into the vagina. The duct forms an effective sphincter controlling the release of stored sperm. After copulation, the bursa contains a mass of living sperm, never oriented or attached to the epithelial cells. There is also a large amount of prostatic secretion which may persist for a time after the sac has emptied of sperm. The lining epithelium (Fig. 10 C) is composed of simple columnar cells of uniform

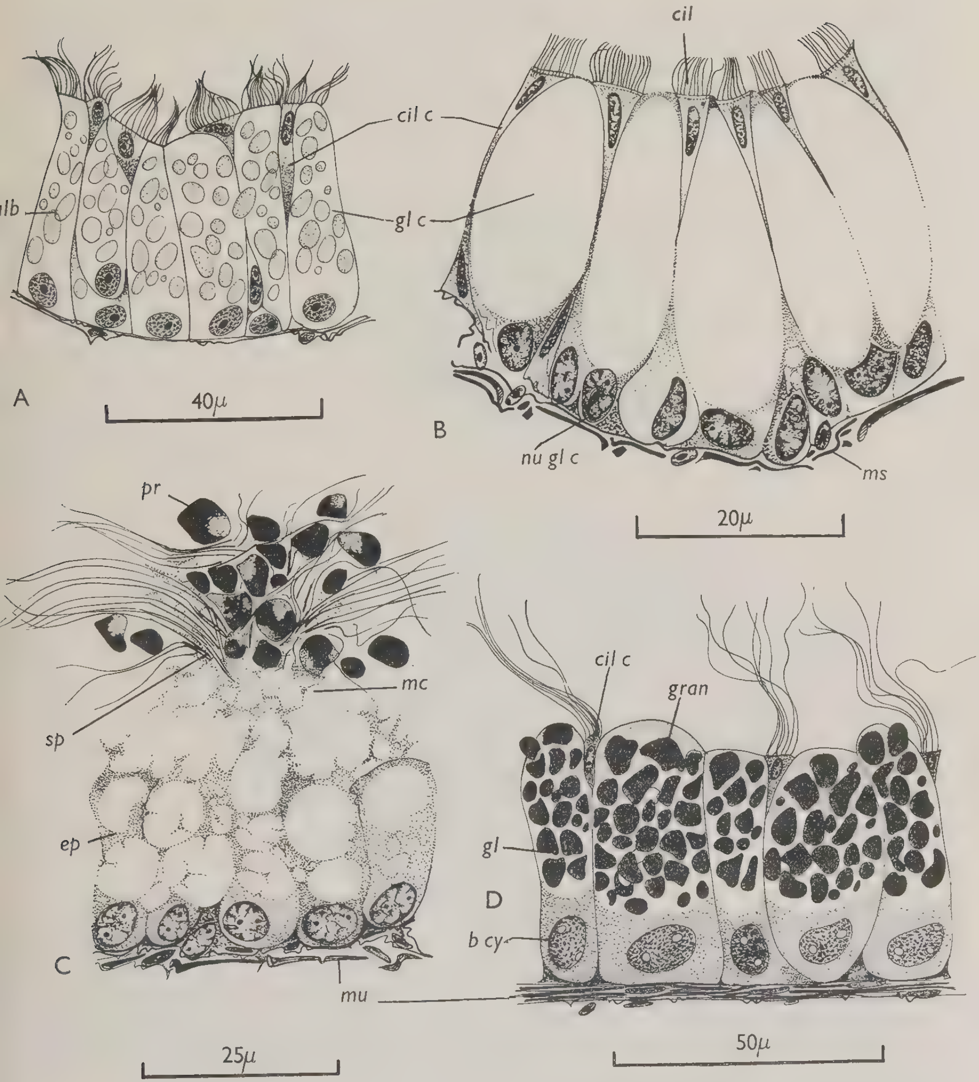
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Fig. 10. *Otina otis*. A: histology of the albumen gland (Bouin's; azan). *alb*, droplets of albumen secretion; *cilc*, ciliated cell; *glc*, gland cell. B: histology of the posterior mucus gland (Bouin's; Masson's trichrome). *cil*, cilia; *cilc*, ciliated cell; *glc*, gland cell; *ms*, muscle fibre; *nuglc*, nucleus of gland cell. C: histology of the bursa copulatrix (Bouin's; Masson's trichrome). *ep*, epithelial cell; *mc*, mucus material of seminal fluid; *mu*, muscle fibre of subepithelial layer; *pr*, granules of prostatic secretion; *sp*, sperms. D: histology of the prostate (Bouin's; azan). *bcy*, denser basal cytoplasm of a gland cell; *cilc*, ciliated cell; *gl*, gland cell; *gran*, granules of prostatic secretion; *mu*, coat of circular muscle.

height (25 μ), without cilia. Secretion does not take place, except by the occasional discharge of cytoplasm when the free surfaces of the cell are ruptured. The nuclei are large (5–6 μ), ovoid to spherical, and lie in an area of denser cytoplasm round the base of the cell. The cell contents are elsewhere vacuolated or reticulate.

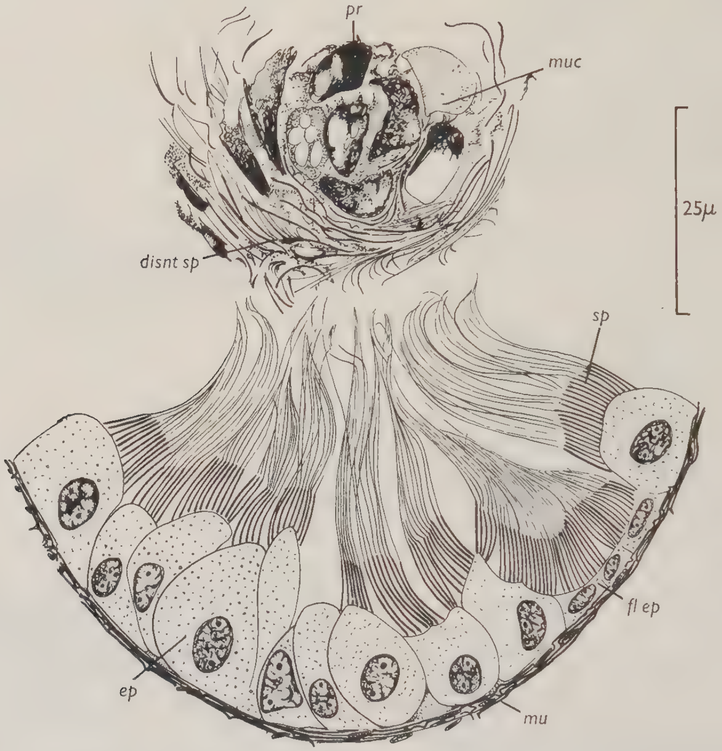

Fig. 11. *Otina otis.* Transverse section of part of the wall of the accessory bursa copulatrix, showing the attachment of sperms to the epithelium. Portion of the mass of disintegrating contents at the centre of the lumen is also figured. *disnt sp*, disintegrating sperms; *ep*, larger, rounded epithelial cell; *fl ep*, flattened or squamous epithelial cell; *mu*, muscle fibres underlying epithelium; *muc*, mucoid secretion, presumably derived from the genital duct; *pr*, granules of prostatic secretion; *sp*, oriented sperms. (Bouin's; azan.)

Arising from the distal common duct, the duct of the accessory bursa copulatrix sweeps across the dorsal surface of the prostate, and passes obliquely backward towards the mid-line. It expands gradually into a terminal sac, 150–200 μ in diameter (Fig. 9, *abs*) which is hidden from dorsal view by an overlying lobe of the digestive gland. The lining epithelium (Fig. 11) is of a rather distinctive kind; the cells may be in places flattened or squamous, especially during distention by sperm, but more often are plump and rounded, bulging freely into the lumen, the largest cells deeply indenting their neighbours. The nuclei are extremely large and rounded, with two nucleoli, central in position or displaced upward by compression. There are no gland cells, but, as in the vaginal bursa, cytoplasm may be released by rupture of the cell walls. From December until May, the accessory bursa is filled with sperm which are attached in clusters to the epithelial cells by their filiform heads. They also become attached to the flattened cells lining the duct, within a short distance of the terminal sac. In the middle of the lumen is usually a compact bolus of disintegrating sperm, appearing pinkish brown through the transparent wall. It consists of sperm heads, intermixed with fragments of cells, and with layers of mucoid and prostatic secretion.

Sperm do not appear to remain long in the vaginal bursa after copulation. Living sperm are evidently stored for some time within the accessory bursa; but whether sperm from this sac travel farther up the genital tract to the fertilization pouch, or whether fertilization is effected by sperm newly released from the vaginal bursa, is still uncertain. Copulation, however, seems to occur several months in advance of oviposition, and the prostate becomes smaller and ceases to secrete by the end of March. An important function of the accessory bursa is the storage and breakdown of surplus sperm. Neither of the sperm sacs in *Otina* appears to be homologous with the receptaculum seminis of the prosobranchs and of *Actaeon* (Guiart, 1901) which lies near the site of fertilization between the albumen and capsule glands. Here sperm is stored immediately after copulation and becomes oriented on the epithelium, and in some prosobranchs it is later resorbed.

Eggs and Development

Otina breeds at Wembury during the latter half of May and the first fortnight of June. The egg capsules are typically found in crevices, on more or less clean rock surfaces, in company with groups of the ovipositing animals. Each egg mass (Fig. 12A) forms a small irregular cluster about 4–5 mm across, loosely attached to the substratum. It consists of a single layer of twenty to thirty eggs surrounded by a tough, translucent mucoid secretion, pale yellow or straw coloured. The eggs are ovoid, 0·3 mm in length, pale yellow in colour. They are surrounded by a layer of albumen, whitish and finely granular, and enclosed in a thin tough capsule, quite smooth and transparent. The egg capsule stains pale blue in azan, as well as black in thionin, identically with the cells of the posterior mucous gland from which it is evidently derived. The investing jelly appears to be the secretion of the anterior mucous gland which is always thick and distended prior to the time of egg laying.

Embryos in eggs were abundant at the end of the first week in June. At liberation (Fig. 12B, C) they are equipped with a wide-throated, trumpet-mouthed shell, quite transparent and with only a few fine growths striae for sculpture. The visceral mass, which is yellow from the large amount of yolk, is contained in the large apical bulb, and the distinctive character of the epithelium of the digestive gland is already partly developed. The disposition of the apical bulb of the shell—on comparison with the same structure in *Leucophytia* (Morton, 1955 *b*)—shows a trace of a brief sinistral phase in the life of the animal. The first half whorl is at right angles to the coil of the rest of the spire, which has a dextral direction. A 'reduced hyperstrophic apex' has been described by Harry (1951) as a typical feature of the basal families of the Pulmonata and Opisthobranchia. It occurs in all the Ellobiidae investigated, with the single exception of the terrestrial *Carychium*, and is present also in the Chilinidae among primitive pulmonates. The possession of such an apex by *Otina* would be one more piece of evidence providing a firm link between this genus and the Ellobiidae at the base of the Pulmonata.

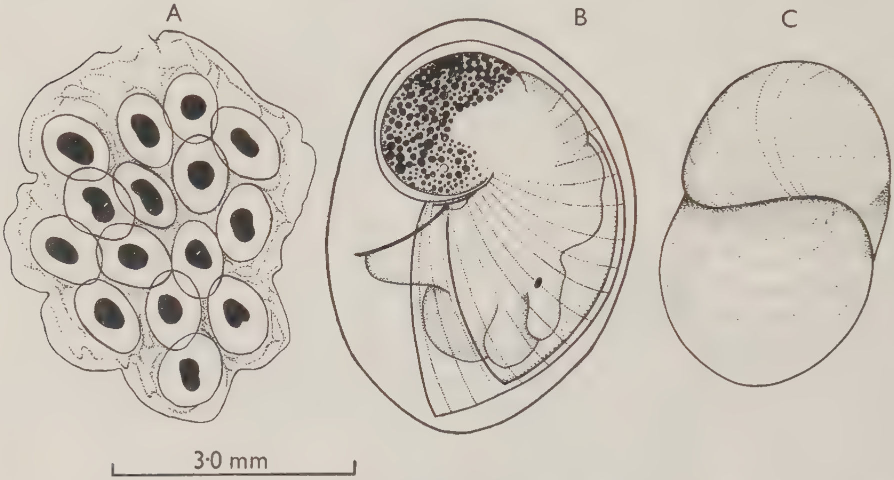

Fig. 12. *Otina otis*. A: portion of an egg mass, shortly after oviposition. B: an embryo ready to emerge from the egg capsule. C: another view of the embryo shell, at the same stage as B.

The tentacles at this stage are short, blunt and club-shaped, the eyes at their bases. The foot bears a transparent chitinous operculum which is not large enough to close the aperture but is used by the emerging animal in thrusting through the capsule membrane. There is no trace of velar lobes nor any detectible velar cilia. The animal upon emergence must at once creep about on the substratum, picking up food by the rasping action of its radula.

Discussion

Though Thiele (1931) regarded the connexion of the Otinidae with the Ellobiidae as strong enough to justify inclusion in a single supra-family, the present family is yet in many respects a rather isolated group. Its main taxonomic resemblances, such as in the pattern of the radula and in the form of the embryo shell are with the ellobiids; but in features less easily accessible to the systematist, such as the structure of the digestive system, and the arrangement of the successive regions of the genital tract, there is found merely a broad general agreement with few detailed resemblances between the two families. In both groups the basic organization is almost wholly unspecialized; but *Otina* differs from the rather generalized snails included in the Ellobiidae in having acquired a number of secondary specializations fitting it for a more 'difficult' habitat. It is thus much better able than any of the ellobiids to withstand the environment of an open rocky shore, with heavy wave attack, living as it does at the threshold of crevices and creeping out, when the humidity of the atmosphere is high, to an exposed position on the barnacle fringe. The shell is broad, depressed and limpet-like, and able to be clamped tightly against the substratum. The adoption of the limpet-habit has brought with it other simplifications in the arrangement of the viscera within the shell. There is a further adaptation in which *Otina* resembles the limpets, the possession of a powerful buccal mass with a scraping radula (a broad membrane in *Otina*—a narrow ribbon in the true limpets) by which the animal is able to feed on a wave-swept, relatively inhospitable substratum, picking up wave-lodged diatoms and gleaning the smallest threads of filamentar algae.

Some further specializations in *Otina* may be regarded as consequences of the small size of the animal; and in this tendency to secondary simplification, there is a parallel between *Otina* and the small mesogastropod prosobranchs described by Fretter (1948). Thus the pallial cavity is a mere narrow cleft receiving the opening of the renal organ, and lacking entirely any vascularized respiratory surface. The kidney itself is reduced in relative size and lined with a simple layer of unfolded epithelium. A respiratory and an excretory function have apparently been assumed by the lateral lobes of the foot. The haemocoele of the foot forms a wide space, loosely meshed with connective tissue and able to be greatly distended with blood. The epithelium of the lateral lobes is thin, and there is evidently an egress of phagocytic cells from the haemocoele. The method of pedal respiration and excretion is clearly dependent upon the small size of the animal and the relatively large ratio of exposed surface to volume; and the loss of special respiratory organs is probably made possible by the relative inactivity of the animal.

When all of these secondarily specialized features have however been taken into account, there can be little doubt of the truly primitive status of *Otina* among the pulmonates. The digestive and genital systems possess few advanced features; and the nervous system, though it has not been reinvestigated in detail in the present work, provides further evidence of an archaic condition. Pelseneer (1901) described and figured a nerve ring, in which the cerebro-pleural commissures are not apparent, these two ganglia being in close contact, in a way which, as Bargmann (1930) points out, is rare in Pulmonata but represents the usual condition in Prosobranchia. On either side, the parietal ganglia are closely drawn into the nerve ring, the pleuro-parietal commissure being on the right very short and on the left wholly lacking as such. There remains in *Otina* a rather long visceral loop, retaining no trace of torsion, and formed, unlike that of the ellobiids, by the parieto-visceral commissure of each side, leading back to the single visceral ganglion.

Turning to the digestive system, the simple character of the stomach may to some extent be a consequence of the small size of the animal, as well as an indication of the primitive condition of this organ. The absence of the posterior caecum, receiving the major typhlosole, seems—for instance—to be a feature in which *Otina* differs from all of the less specialized genera of the Ellobiidae. The lack of a differentiated muscular gizzard is another primitive negative character, which must be possible in *Otina* only by reason of the fine, well-comminuted nature of the food particles. Adding to the lack of a gastric gizzard the presence of ciliated excurrent grooves from paired digestive diverticula, and of the vestige of an intestinal 'style sac', we may recognize in the otinid stomach a most convincing transitional stage between the condition of the microphagous Prosobranchiata and that of the 'higher' Basommatophora. A 'ballooning-out' of the posterior muscular pocket, and the further strengthening of its cuticular lining, would give to this part of the stomach, in *Otina*, the gizzard-like appearance which is a feature of the ellobiids and of all the other aquatic pulmonates yet investigated.

The structure of the digestive gland agrees with what is known of other primitive pulmonates and of at least some opisthobranchs. The glandular epithelium undergoes a cycle of ingestion, excretion and fragmentation. At the last stage it would appear that 'fragmentation phagocytes' become abstricted from the digestive cells, to wander into the stomach where they carry out a non-localized absorptive and digestive function. The occurrence of such a digestive gland, with the fragmentation of its absorptive epithelium, seems to be correlated in pulmonates and opisthobranchs with the loss of the extensive gastric sorting area of the prosobranchs. Amoebocytic cells, of the type which primitively wandered into the lumen of the gut from the blood vessels underlying the sorting area, are now replaced by 'phagocytes' originating directly from the digestive epithelium.

The reproductive system, like the stomach, remains at a primitive level in *Otina*. An archaic feature, which is shared by most of the ellobiids, is the open communication between the male and female regions of the glandular genital tract. Further, there are indications that at a recent time in phylogeny the male and female genital apertures were widely separated, the female aperture near the pallial opening and the (secondary) male aperture far forward on the head, as is still found in the ellobiids and in all tectibranchs. In *Otina* these two apertures have now moved secondarily close together, both lying beneath the right oral lappet. From the original common genital aperture, giving rise to the definitive female opening, the secondary male aperture must first have been carried forward to the head, by the usual shallow extension of the vas deferens, forming an open groove in tectibranchs and a closed tube just beneath the integument in pulmonates. In *Otina*, apparently uniquely, the female aperture has been carried anteriorly from its pallial position in the same manner, by the prolongation of the vagina as a narrow muscular tube, closely accompanying the vas deferens.

The albumen gland in *Otina*, like that of the ellobiids, but unlike the same gland in the actaeonids, has lost its primitive pallial position and has entered the haemocoele, where it no longer forms a tubular section of the genital duct traversed by the eggs, but serves as a glandular annexe communicating rather narrowly with the mucous gland in the neighbourhood of the fertilization pouch. In the male portion of the glandular hermaphrodite duct, the prostate has its characteristic pulmonate position, incorporated in the main course of the genital tract, rather than—as in opisthobranchs—forming a glandular diverticulum of the male duct. The absence of a well-developed muscular penial papilla, and the functioning of the preputium as a 'pseudopenis' must probably be regarded as a specialization due to small size.

Most of the earliest pulmonates, as characterized by the ellobiids, retain a heterostrophic embryonic shell, held to be a common inheritance from the ancestral stock of both pulmonates and opisthobranchs (see Morton, 1955*b*). In *Otina* a reduced heterostrophy is still to be seen in the shell of the embryo. Such a condition resembles that of *Leucophytia* and other ellobiids mentioned by Harry (1951) and the pyramidellids (Fretter & Graham, 1949). In one ellobiid genus, *Melampus*, a well-developed velum is retained by a free-swimming larva, and in *Leucophytia* a large vestige of the velum survives in the unhatched embryo. In the embryo of *Otina* there is no trace of a velum. An operculum however persists in this, and in all ellobiid embryos studied.

A characteristic feature of pulmonates at a basal level of organization is the occurrence of a protandrous sexual succession. Such a phenomenon has already been described in *Leucophytia* and *Carychium*. A similar month-by-month survey of the condition of the gonad in *Otina* revealed an extremely close similarity to that of *Leucophytia*. It should again be pointed out that a clear-cut separation of the sexual stages with complete separation of male and female phases is never obtained. From September to December, while sperms are undergoing maturation in the gonad, small oocytes are already prominent in the germinal epithelium, and the female portions of the glandular genital duct are already fully differentiated, though of very small size. The period of female development, between December and June, is marked by the great increase in the relative size of the albumen and mucous glands, and the reduction in size of the prostatic epithelium. The earlier pulmonates thus show two sexual stages in a single season. A question not finally determined is whether *Otina* is an annual mollusc, or whether a restitution of male germinal epithelium is possible following the deposition of eggs. From the fact that animals of the whole size range were evidently equally abundant at all times of the year, it would seem that *Otina*, like the majority of pulmonates, undergoes at least a biennial life cycle. An opposite example is that of *Skeneopsis*, a minute prosobranch, investigated by Fretter (1948) and found to have an annual life cycle, with a great falling off in the numbers of adults during winter months.

The limpet habit in the Basommatophora has been independently developed at least four times, and is associated either with resistance to wave attack on the seashore or with life in fast-flowing inland waters. With the acquisition of its 'lung', *Otina* must have evolved in a way similar to the larger limpet-like marine pulmonates, the Siphonariidae. The latter family has undergone a parallel reversion from the upper shore to a completely intertidal life (see the monograph by Hubendick, and also the recent papers of Yonge (1952) and Borland (1950). We may suppose that at the level of the origin of the first pulmonates the lung was acquired as part of the heritage of a primitive stock of supratidal gastropods. There are many examples, however, of a return to aquatic life, and among the lower Basommatophora, most of the families are characterized by an early retreat to the sea. Perhaps they never reached far beyond the shore, but acquired the lung—as, for example, in the Ellobiidae and the Amphibolidae—as an adaptation to easier aerial respiration in the turbid, oxygen-poor water of estuaries. Such pulmonates stand rather apart from the four families of the higher 'limnic Basommatophora' (the Lymnaeidae, Physidae, Planorbidae and Ancylidae). Their embryos carry opercula, and most of them bear vestiges of a velum. They retain as a mark of primitiveness a heterostrophic apex, and a relatively long visceral loop. The Siphonariidae are known to have arisen very early, according to Zittel (1923) in the Devonian. Probably the other families of primitive Basommatophora spring from an equally ancient stock; and in a reconsidered arrangement of the Pulmonata these marine families, with probably the Latiidae and Chilinidae, might be placed together in a natural assembly of 'Archaeopulmonata'.

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UPTAKE OF RADIOACTIVE SODIUM (^{24}Na) BY *NEREIS DIVERSICOLOR* MUELLER AND *PERINEREIS CULTRIFERA* (GRUBE)

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(Text-figs. 1–4)

INTRODUCTION

Nereis diversicolor is typically euryhaline: it is in equilibrium with normal sea water (Schlieper, 1929; Beadle, 1937), and develops hypertonicity in more dilute media which it is able to maintain indefinitely. The maintenance of this steady state is preceded by a transition period. During this there is a rapid uptake of water accompanied by a loss of salts from the worm, resulting in a fall in the osmotic pressure of the body fluids, and then a subsequent water loss which, according to Ellis (1937), is not accompanied by an uptake of salts. Worms accommodated to 25% sea water weigh about 140% of their original weight (Beadle, 1931). Beadle (1937) found that when the steady state is attained the body fluid concentration in 50% sea water approaches a value which is about 5% higher than that of the external medium, whereas in 25% sea water the concentration of the body fluid is equivalent to about 44% sea water. His worms were collected from the Northumberland coast, and concentration measurements of the body fluid were determined by Baldes's modification of the Hill vapour-pressure method. The results of Schlieper (1929) on worms from Heligoland, where they may live in a salinity as low as 4‰, are based on freezing-point determinations and give a higher internal concentration in the dilute media: after 3 days in 50% sea water the concentration of the body fluid is equivalent to 68% sea water, and after 2 days in 25% sea water equivalent to 49% sea water. These varying results suggest the occurrence of local physiological races.

The method by which this hypertonicity is maintained is open to conjecture. Since the concentration of the body fluids is increased when an animal previously treated with dilute sea water is transferred to an isotonic solution, Beadle (1937) concludes that the mechanism must entail an active process which is not merely controlling the inflow of water. He cites three possibilities: the excretion of a hypotonic fluid by the nephridia; the addition of salts to the body fluids from the tissues, or, as in *Carcinus* (Webb, 1940), an active uptake of salts from the external medium. It is, of course, probable that more than one of these mechanisms is involved. Beadle favours the first and states that the most reasonable conclusion is that the animal behaves as a normal osmotic system as regards uptake of water and change of internal concentration, but that the volume of the body fluids is continually being reduced by the removal in the nephridia of a fluid hypotonic to the body fluid. In a previous paper Beadle (1931) showed that the respiratory and the weight curves of an animal in dilute sea water are of the same form: they each have an approximately simultaneous maximum and a subsequent fall. He suggests that the extra oxygen consumption is not the result of osmotic work, but rather that of work done by the body-wall muscles in resisting swelling. That hypertonicity is maintained, at least in part, by the active uptake of salts from the environment is, however, suggested by the results of both Schlieper (1929) and Beadle (1937).

The uptake and loss of chloride by an organism is easy to demonstrate. It has been studied for a number of invertebrates as well as anamniotes. Ellis (1939) measured the chloride lost by *Nereis diversicolor* and *N. cultrifera* when placed in 20% sea water, and also its uptake when the worms are transferred back to 100% sea water. Isotopic indicators which are now in use give further scope for studying the passage of ions between an aquatic organism and its environment. By this means Jørgensen, Levi & Ussing (1947) have recorded the uptake of $^{24}Na^+$ and $^{38}Cl^-$ by the axolotl, and Abelson & Duryee (1949) the exchange of radioactive sodium by the frog's egg. Jørgensen & Dales (1954), by using the tracer $^{36}Cl^-$, have shown that at certain dilutions of the external medium there is an active uptake of chloride ions by *N. diversicolor* and *N. virens* which have been adapted to the diluted medium, and so are in a steady state with respect to their chloride content.

METHODS

The tracer element ^{24}Na with a half-life of 14·9 h allows only short-term experiments to be carried out. It is clean to work with, however, and the slight amount adsorbed on to the surface of an organism is readily removed by washing in water. Sodium comprises the predominant cation of the environment of a marine animal, and the experiments of Ellis (1937) have shown that in *N. diversicolor* it is essential in the weight regulation associated with osmotic control. The uptake of the element by a living organism can be followed by using the apparatus designed by Arnott & Fossey (1952). It consists of 8 G.M. Tubes (20th Century, G 10 P6) set in a ring around a Perspex tube and connected in parallel. A second Perspex tube surrounds the counters and the whole is shielded by a lead castle, which can be opened at the top to the inner Perspex tube. For the present experiments a disc of sheet cork, with a hole centrally placed, was fitted horizontally into the top of the inner Perspex tube, and through the hole was slipped a boiling tube: the rim of the tube is held by the cork and the rest of it, which may contain the animal to be assayed, is exposed to the counters. A similar use of γ-Müller tubes as a means of assaying different sources of radioactivity has been described by Freedberg, Ureles & van Dilla (1949) and Veall & Vetter (1952).

^{24}Na obtained from A.E.R.E. Harwell as $^{24}Na_2CO_3$ has the high specific activity of 32 mc/g. Tracer amounts never greater than 16 mg were added to each litre of sea water, which raises the sodium content by a maximum of 0·0148%. Each experimental worm was in 100 ml. of activated water in a darkened jar: the jar was shielded at the opening, and the water kept saturated with oxygen at normal atmospheric pressure. Experiments were maintained at a constant temperature, usually 14° C; others were at 5° C. Sea water used for the experiments had a salinity of 35‰. Its sodium was labelled with ^{24}Na, and the activity of 1 ml., indicating the presence of 10·8 mg of inactive sodium, was recorded at the beginning of the experiment. Such a standard was used to calculate the uptake of sodium by a worm under specific experimental conditions. Distilled water was added to the sea water to give the various dilutions, and hypertonic sea water was obtained by slow evaporation of the normal sea water at a temperature not higher than 60° C.

UPTAKE OF SODIUM BY *NEREIS DIVERSICOLOR* AND *PERINEREIS CULTRIFERA* IN WATER OF VARYING SALINITIES

The uptake of sodium by *Nereis diversicolor* Müller was first studied on worms which were accommodated to the various salinities of sea water to be used in the experiments. These animals would be in a steady state with respect to their sodium content. Prior to the experiment the wet weight of each worm was recorded, and those weighing about 1 g were chosen. These worms were then placed in sea water of a salinity of 35, 33, 17·5, or 9‰, or in hypertonic water, which was kept saturated with oxygen at normal atmospheric pressure and was at a constant temperature of 14° C. The weight of all these individuals had reached a relatively steady value at 36 h (Beadle (1937) found that the regulation of the osmotic pressure is completed in advance of volume regulation and well within this time). Each animal was then dried on filter-paper, the weight recorded, and it was placed in the experimental vessel which contained sea water of the same salinity as that from which it had been taken, but with the tracer sodium added. After a period ranging from 1 to 2 h the worm was removed from the vessel, washed rapidly in three changes of sea water and presented to the counting apparatus. For animals in dilute sea water the uptake of sodium per g wet weight was calculated on the original weight of tissue, the osmotic uptake of water being neglected; allowance was made for the slight loss of weight due to starvation.

The results of such an experiment are shown in Fig. 1. In sea water of a salinity of 35‰ the uptake of sodium per hour ranges from 275 to 240 μg/g wet weight; the higher figures appear to be associated with the more active worms. In a salinity of 17·5‰ the uptake per hour reaches an average of 161 μg/g wet weight, which is rather more than might be expected if the worm behaves as a simple osmometer; in water of about half this salinity (9‰) the uptake may even exceed this figure, reaching an average of 180 μg/g wet weight. In lesser dilutions, as in a salinity of 33·5‰, the sodium influx is proportional to the degree of dilution, indicating a passive exchange of ions. For worms adapted to hypertonic sea water of a salinity of 43‰ the uptake is slightly less than might be expected. The hypertonicity of worms in brackish water may involve the uptake of ions in different proportions from the normal, since Cole (1940) found a differential accumulation in *Homarus* in similar conditions.

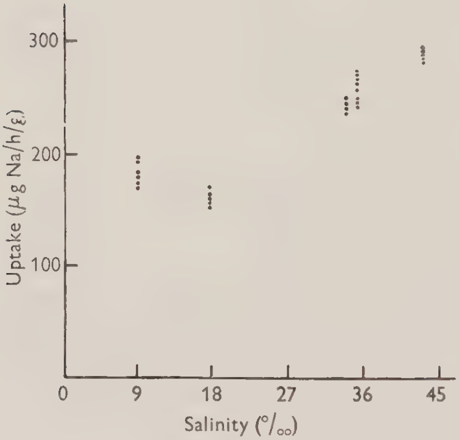

Fig. 1

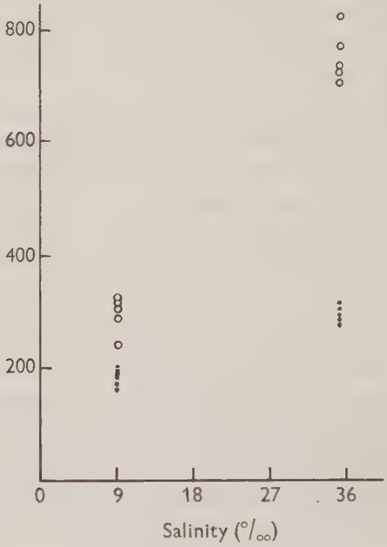

Fig. 2

Fig. 1. *Nereis diversicolor.* Rate of uptake of sodium in sea water of different salinities. (Rate expressed as micrograms of sodium per hour per gram wet weight.)

Fig. 2. *Perinereis cultrifera* and *Nereis diversicolor.* Rate of uptake of sodium in sea water of 9‰ and 35‰ salinity. Rings indicate results for *Perinereis* and dots for *Nereis.*

Jürgens (1935) has shown that respiration takes place through the epithelium of the gut of *Nereis diversicolor* as well as through the integument. It may be that the gut wall can serve as an area of active absorption of ions for a worm in a hypotonic medium. To test this the previous experiment was repeated with a series of animals which had the body tied anteriorly and posteriorly with nylon thread to close the passage to the gut, and another series acted as a control. Both sets of worms were transferred from normal to diluted sea water, some in 50% and others in 25%, and they were weighed at intervals for a comparison of the weight regulation. When accommodation was completed the uptake of sodium was measured. The results (Table I) showed that the weight regulation of worms which had the gut obstructed, and were in

50% or 25% sea water, followed the normal course; and the uptake of sodium by these individuals showed only slight individual variations from the results of the control specimens.

These results suggest that only the integument is concerned with the uptake of salts, or that, when necessary, the integument can compensate for the loss of the gut surface as an area of transport to and from the body tissues.

TABLE I. UPTAKE: μg Na/h/g WET WEIGHT

	Salinity	
	9‰	17·5‰
Gut obstructed	180	175
	150	160
	170	140
Controls	183	164
	200	150
	170	170

The uptake of sodium from 100% and 25% sea water was studied for *Perinereis cultrifera* in the same way as for *Nereis*. *Perinereis* is a more typically marine form, and although it can withstand a lowering of the salinity of sea water, its weight curve in brackish water increases to a higher value than that of *Nereis diversicolor*, and there is no subsequent fall. In 25% sea water the animal becomes sluggish. The results of an experiment in which both polychaetes were used for comparison are shown in Fig. 2. From these it can be seen that *Perinereis* is the more permeable to sodium ions: in normal sea water the uptake is nearly 3 times greater than in *Nereis*, and in water of about a quarter this salinity the uptake is at least 1·5 times as great. In the dilute medium even this species takes from the environment a relatively greater amount of sodium than worms in normal sea water: Wells & Ledingham (1940) state that in 25% sea water *Perinereis cultrifera* maintains a slight degree of hypertonicity. The lower permeability of the integument of *Nereis* must be of importance in diminishing the amount of work necessary to maintain osmotic independence. These results, however, are contrary to those of Ellis (1939). In measuring the chloride output of *N. diversicolor* and *Perinereis cultrifera* in 20% sea water he records a higher loss of chloride from the former than from the latter, and suggests that *Nereis diversicolor* swells less in dilute sea water because of the more rapid loss of salts relative to water intake.

The influx of ions to the tissues of worms which are transferred from dilute to normal sea water is high, and is approximately inversely proportional to the dilution of the medium from which they are taken. To estimate this the following experiment was carried out. The exchange of sodium per hour by twelve specimens of *N. diversicolor* which had been living in sea water of a salinity of 35‰ was calculated, and these individuals were then transferred to a more dilute medium, four worms being placed in water of each of the following salinities, 27, 17·5 and 9‰. After 40 h they were returned to normal sea water containing ^{24}Na and their uptake of sodium per hour was again measured—when returned to normal sea water the weight of the worm is reduced with the loss of water. The results are shown in Fig. 3, and the results of a similar experiment for a comparison between *N. diversicolor* and *Perinereis cultrifera* are given in Table II.

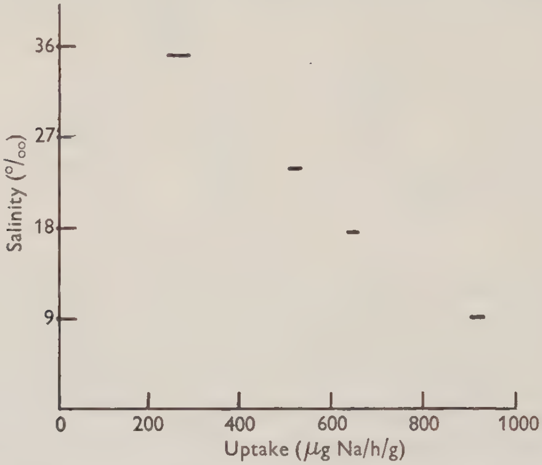

Fig. 3. *Nereis diversicolor.* The thickened bar at 35 ‰ salinity is the range of the rate of uptake of sodium per hour (per gram wet weight) for twelve worms. At the other three salinities is similarly shown the range of uptake by four worms when returned to sea water of 35 ‰ salinity from brackish water.

TABLE II. μg Na/h/g WET WEIGHT OF WORM

	Uptake per hour in normal sea water (salinity 35 ‰). Average of four worms	Uptake per hour on return to normal sea water after 26 h in dilute sea water (salinity 9 ‰). Average of four worms
N. diversicolor	260	969
P. cultrifera	720	2960

The increased uptake on return to normal sea water presumably compensates for the loss of salts in the lower salinities. The increase is about 3 times greater for *P. cultrifera* than for *Nereis diversicolor*.

The apparatus which has been used for these experiments makes it possible to follow the rate of uptake of radioactive sodium to the level at which equilibrium is reached, and the amount of the tracer element in the worm ceases to increase. From this level can be determined the total amount of exchangeable sodium. For *N. diversicolor* accommodated to water of a salinity of 9‰ this is approximately 3·2 mg/g wet weight. For worms living in a salinity of 17·5‰ there may be only a slight increase over this amount, though it is doubled when the animals are in sea water of full salinity (35‰) and for an average of twenty individuals was 6·3 mg/g wet weight. There would thus appear to be a correlation between the osmotic pressure of the body fluid and the amount of exchangeable sodium. In hypertonic sea water the amount increases: for the average of six worms in water of a salinity of 51‰ it reached a value of 7·2 mg/g wet weight. *Perinereis* has a higher permeability than *Nereis*, yet the total amount of exchangeable sodium per g wet weight in 100 and 25% sea water is slightly less than for *Nereis*.

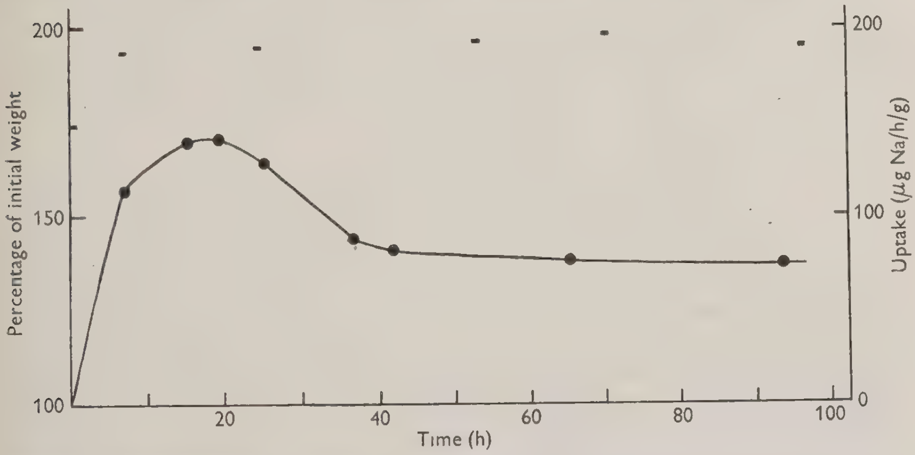

Fig. 4. *Nereis diversicolor*. Uptake of sodium per hour (per gram wet weight) during the period of weight regulation when transferred from sea water of 35‰ salinity to that of 9‰ salinity. Continuous line indicates weight regulation.

UPTAKE OF SODIUM BY *NEREIS DIVERSICOLOR* DURING THE PERIOD OF ACCOMMODATION TO DILUTE SEA WATER

No study has yet been concerned with the uptake of ions by *N. diversicolor* during the period of osmotic swelling and subsequent weight adjustment in water of low salinity. For this purpose freshly collected specimens were kept for a period of 36 h in normal sea water (35‰). Twenty worms were then weighed individually and each isolated in a vessel containing water of a salinity of 9‰. At a temperature of 14° C the typical weight curve followed the course shown in Fig. 4. At varying intervals of time along this curve certain worms were placed in activated sea water for an hour or two, so that an estimate of the sodium uptake at that point in the weight regulation could be made. Afterwards they were returned to the inactive water. The handling of worms in this way did not appear to have any adverse consequences. The results show that throughout the period of osmotic adjustment, which involves varying volume changes, there is an active uptake of sodium from the brackish water at an approximately constant level. The only variation is at the beginning of the experiment when it is lower, though not sufficiently low to suggest that there is any serious time lag in the establishment of the active uptake. An experiment for worms transferred from water of a salinity of 35‰ to that of 17·5‰ gave similar results.

Thus it seems that, as in *Eriocheir* (Krogh, 1939), the passage of ions through the integument is unrelated to the passage of water, though the two events take place simultaneously. This suggests two independent transport processes. The rapidity with which the active uptake of ions is established when the worm is placed in dilute sea water may mean that chemoreceptors in the integument are influenced, and regulate the activity of the ion-transporting cells through nervous or hormonal activity. Or changes in the concentration of ions may act directly upon the ion-transporting cells and induce appropriate changes in uptake.

EFFECT OF TEMPERATURE AND OXYGEN DEFICIENCY ON THE UPTAKE OF SODIUM

All results which have been quoted concern experiments in which worms were in water of a constant temperature, and saturated with oxygen at normal atmospheric pressure. This is necessary for comparative results. Beadle (1931) investigated the effect of oxygen lack on the weight curve of *Nereis diversicolor* in 25% sea water, and found that the weight rises to a higher value than when oxygen is present, approaching the condition of the normal weight curve of *Perinereis cultrifera* when transferred to 25% sea water. Moreover, there is no subsequent loss of water from the tissues, which suggests that this is an active process carried out only in the presence of oxygen. Oxygen deficiency also has an effect on the uptake of sodium ions. When *Nereis* is taken from normal sea water and placed in brackish water with a low oxygen content—water through which nitrogen has been passed, or even water which is not kept saturated with oxygen—the rate of active uptake of sodium is above the normal, and the total amount of exchangeable sodium increases; these differences become greater with decreasing salinity. For a worm taken from 100% and placed in 25% sea water the influx in 20 h is twice as great as when the water is saturated with oxygen: for a worm already accommodated to 25% sea water, and therefore with a lower oxygen consumption, it is less.

A low temperature also influences the weight adjustment of worms which are transferred from normal to dilute sea water. Beadle (1937) working with *N. diversicolor* collected from the estuary of the River Blyth, Northumberland, found that 'during the winter months the increase in weight in 25% sea water was greater and more prolonged than during the summer', though his graph shows the reverse of this. He does not, however, give the winter temperatures. Results of my experiments carried out at 5° C agree with his statement. At 5° C the weight curve for animals placed in 25% sea water rose slowly during 24 h to over 1·8 times the weight in normal sea water, which exceeds the weight increase at 14° C, and it showed no reduction for the following 24 h. At all salinities the penetration of sodium was slower at this lower temperature. Particularly noticeable is the fact that worms in 25% sea water, where more osmotic work is needed, take up less per h per g wet weight than those in 50% sea water. It is known that high temperature reduces the degree of hypertonicity which must be maintained by an animal for survival in dilute media. Pannikar (1940) suggests that it is for this reason that the most active colonization of fresh and brackish water by marine animals takes place in the tropical areas. May this not also be due to the fact that the processes concerned with osmoregulation are carried out more speedily at higher temperatures and so are less impedient?

This work was carried out at the Plymouth Laboratory of the Marine Biological Association, and was aided by a grant from the Browne Research Fund of the Royal Society. My thanks are due to members of the Plymouth Laboratory for their help, and to the Council of the Royal Society.

SUMMARY

In sea water of a salinity of 35‰ saturated with oxygen at normal atmospheric pressure and at a temperature of 14° C, the uptake of sodium by *Nereis diversicolor*, which is in equilibrium with its environment, ranges from 275 to 240 μg/h/g wet weight. The higher uptake appears to be associated with the most active worms. When the salinity is reduced by half the uptake, for worms accommodated to this dilution, averages 160 μg/h/g wet weight, and at 9‰ it averages 180 μg, which shows that there is an active uptake of salts against the concentration gradient. For a salinity slightly higher or lower than that of normal sea water the influx of the salt is approximately proportional to the degree of dilution or concentration. The closing off of the intestine has no effect on the rate of uptake.

The integument of *Perinereis cultrifera* is more freely permeable to ions than that of *Nereis diversicolor*, the influx of sodium being about 3 times greater in normal sea water, and at least 1·5 times as great in 25% sea water. When worms are transferred from dilute to normal sea water the uptake of sodium is high compensating for the loss of salts; it is about 3 times greater in *Perinereis* than in *Nereis*.

For *Nereis* in sea water of salinity 9‰ the total amount of exchangeable sodium approaches 3·2 mg/g wet weight: in normal sea water it is about double this amount.

During the period of accommodation to dilute sea water by *N. diversicolor* there is an active uptake of sodium, which remains at an approximately constant level throughout the period of weight fluctuation, and is of

approximately the same value as for worms already in equilibrium with their environment.

Oxygen deficiency increases the rate of uptake from dilute sea water and also the total amount of exchangeable sodium. A low temperature (5° C) causes a greater imbibition of water during the period of accommodation to dilute sea water, it slows down the weight adjustment and reduces the rate of uptake of sodium.

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THE SUBLITTORAL FAUNA OF TWO SANDY BAYS ON THE ISLE OF CUMBRAE, FIRTH OF CLYDE

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(Text-figs. 1 and 2)

INTRODUCTION

There is a considerable literature on the ecology of intertidal animals and a growing one on the sublittoral fauna. Largely because of the difficulty of taking samples in very shallow water, little attention has been paid to the continuation of the intertidal zonation below the low-water mark. Although incomplete in some respects, the results of the present survey are published, partly to help bridge the gap between studies of sublittoral and intertidal faunas, partly because it is unlikely that this survey will ever be completed and partly because the intertidal fauna of one of the bays is particularly well known. The work was begun in 1938 by one of the authors (A.M.) but was discontinued at the outbreak of the late war. Since 1949 further collections have been made and the identity of most of the species taken in the earlier sampling has been checked. The collections of animals and a full account of the results have been deposited in the Marine Laboratory at Millport.

METHODS

None of the larger and more reliable bottom samplers can be operated from the small boat that must be used in shallow water. All samples in the quantitative survey were taken by the Robertson mud bucket, that is, a cylindrical bucket about 15 in. long and 8 in. in diameter with a sharpened edge. The bucket lies on its side and, when hauled, cuts downwards at an angle until filled. The volume of the material taken in a sample is about 8000 ml. This instrument has several advantages; it can be used from a rowing boat (with or without an out-board motor) in very shallow water, it fills rapidly even when the substratum is hard sand and, so far as one can see on a calm bright day, the bucket fills in the same way at all depths within the limits of visibility, irrespective of the angle of the hauling rope. One disadvantage is that it collects a disproportionately large number of animals living just below the surface of the sand, though this drawback it shares with the majority of bottom samplers in current use. Another disadvantage is that densities of populations cannot be expressed in absolute terms, i.e. numbers per unit area of sea bed. However, the Robertson bucket does give reasonably accurate estimates of relative densities and that is sufficient for the purposes of this paper.

Each sample was washed through a 2 mm sieve. From the residue the larger animals were picked out by hand, while a binocular microscope was used to search for smaller animals. Formalin was added to dislodge small animals, such as *Siphonoecetes* which live in crevices of stones and shells.

A series of ten samples was taken at each of seven stations at depths of 1, 3, 5, 6½, 10½, 20 and 27 m below low water (mean ordinary tide) in each of the two bays. Kames Bay was studied in late autumn and winter (between November 1938 and January 1939) and White Bay in late spring and summer (between May and August 1939). This difference in sampling time may affect comparisons of densities for the two bays in the case of species whose young appear on the sea bottom in the early part of the year. It is impossible to say which bay is favoured in this respect: on the one hand, some young will not be large enough for retention on the sieve by May–August; on the other hand, some young will have succumbed from natural causes before November–January. In the preliminary work of 1938–39, the prime concern was to establish the composition of the fauna in the two bays and gain facility in identification. For the comparison proper, it was intended to repeat the sampling simultaneously in the two bays at the rate of 30–50 buckets per station in the winter of 1939–40. Unfortunately the Second World War frustrated this intention.

In order to collect animals living on, or just above, the surface of the substratum, samples were taken by a 4 ft. 9 in. beam trawl with a ¼ in. mesh lined with 2 mm stramin. The trawl was hauled for a standard distance at each station in calm weather.

CONDITIONS IN THE TWO BAYS

The two bays studied were Kames Bay, facing south-east and sheltered from all other directions, and White Bay, facing north-north-east and relatively exposed to the east and north. The Isle of Cumbrae is exposed to the open sea to the south but is sheltered to some extent by neighbouring islands and the mainland in other directions. In Kames Bay the substratum consists of sand grading into fine mud at the deeper stations with only a slight admixture of stones, while White Bay is consistently coarser with a much higher proportion of stones and shells at each station. One peculiar feature of Kames Bay is the large amount of vegetable debris, consisting of fragments of algae and land plants, which lies on the bottom and is washed backwards and forwards with the tide. Although a certain amount of this debris is found at all stations, it is most plentiful at the three shallowest stations. Some idea of the movement of this debris can be gathered from the volume of debris retained by a stramin covered trawl dragged over the sea bed for a standard distance at each of the three inshore stations:

Station ...	1	2	3
Low water, 15 March, 3 p.m.	27	4	2
High water, 16 March, 10 a.m.	4	2	2

(Arbitrary units of volume)

At high water the bulk of the debris is over the intertidal region of the shore. The debris carries its own fauna, particularly of crustaceans, which does not figure in the bottom samples, but must nevertheless play an important role in the economy of the infauna both in competing for and providing food.

There is also a considerable volume of debris in deeper water around station 7 and possibly extending beyond it. It is of a different character from the loose debris washed backwards and forwards by the tide in that it forms the permanent superficial layer of the substratum. Usually it is partially decomposed. The volume of debris collected by the trawl at high tide when most of the movable debris is above station 1 and in the intertidal zone is given below.

Station ...	1	2	3	4	5	6	7
Volume of debris	143	8	25	4	80	300	15,000–20,000

(Arbitrary units of volume)

The Fauna of Kames Bay

The collections made in Kames Bay supplement and extend those made by Elmhirst (1931), Stephen (1928, 1929, 1930) and Watkin (1942), who, between them, have described in considerable detail the zonation of animals in the intertidal part of the bay. The lowest station of Stephen (1928) corresponds approximately with the highest station in the present survey. Elmhirst (1931) also studied the zonation of the Crustacea in the upper part of the sublittoral zone. The position of the stations is illustrated in Fig. 1.

Polychaeta

The distribution of the more common polychaetes is given in Table I. On comparison with the data given by the earlier authors for the intertidal zonation, it will be seen that in no case does a species occur in large numbers both above and below the low-water mark. This discontinuity is not found in the other groups. *Nephthys hombergii* is numerous sublittorally and indeed is common in muddy deposits at all but the greatest depths throughout the Firth of Clyde. Yet although it appears in moderate numbers in the intertidal zone of other parts of the Clyde (Stephen, 1928), it does not do so in Kames Bay (Stephen, 1930; Watkin, 1942). According to these authors, *Nephthys caeca* is common in Kames Bay down to low tide level but it has not been recorded at all from the sublittoral of this bay. *Spio filicornis* and *Phyllodoce* *maculata* are common at station 1, very rare below that, and absent altogether above low tide level. *P. groenlandica* replaces *P. maculata* above low-water mark, but it is not nearly as common. Most species which appear to reach a maximum density at station 7 are, of course, more numerous still in deeper water, e.g. *Scalibregma inflatum*, *Notomastus latericeus*, *Lumbrinereis hibernica*, *Lipobranchius jeffreysii* and *Glycera rouxii*. The last three species are well represented in muddy deposits at almost any depth throughout the Clyde Sea area. By contrast, *Melinna palmata* appears suddenly and in very large numbers at station 7 and seems to occupy a narrow belt at a depth of about 30 m in several sandy bays in the area.

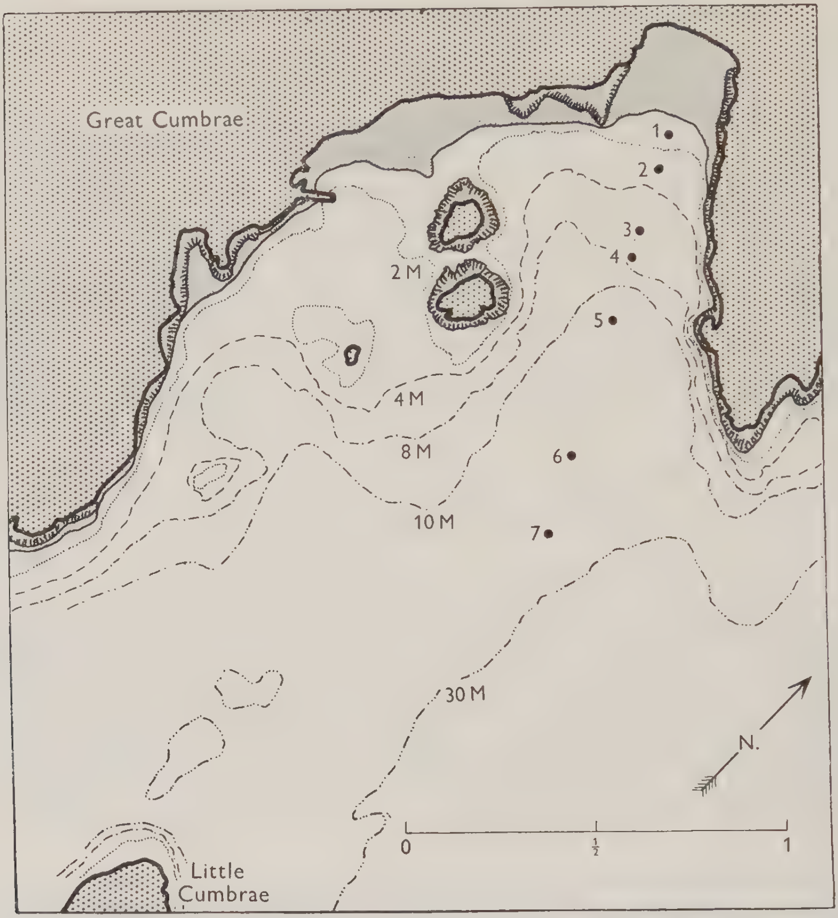

Fig. 1. Kames Bay, Isle of Cumbrae, showing the position of the stations at which samples were collected.

Amphipoda

Elmhirst (1931) has studied the distribution of crustaceans in Kames Bay from the high-water mark to a depth of 6 m below low water, spring tide. The present survey therefore overlaps Elmhirst's. In the overlapping region it confirms him, and his picture of zonation can now be extended to a depth of about 30 m. Only five species of amphipod are numerous below low-water mark (Table II). *Bathyporeia guilliamsoniana* appears in greatest numbers about low tide level and extends for a short way into the sublittoral. In deeper water *Iphinoë trispinosa* and *Siphonoecetes dellavallei* take its place. *Ampelisca brevicornis* (*A. laevivata* of Elmhirst) occurs in small numbers from station 3 to station 7 with a small maximum at stations 3 and 4, while *A. tenuicornis* largely replaces it at stations 6 and 7 and extends into deeper water still.

TABLE I. DISTRIBUTION OF POLYCHAETES IN KAMES BAY

Station ...	1	2	3	4	5	6	7
Nephthys hombergii	92	85	42	19	43	31	45
Spio filicornis	70	—	2	—	2	—	—
Phyllodoce maculata	23	—	—	1	1	—	—
Sigalion mathildae	2	37	41	73	29	—	—
Owenia fusiformis	—	—	9	88	89	79	117
Sthenelais limicola	—	2	1	7	2	1	—
Goniada maculata	—	—	—	2	—	82	71
Stylarioides plumosa	—	—	—	—	—	15	5
Platynereis dumerilii	—	—	—	—	1	37	4
Scalibregma inflatum	—	—	—	—	—	19	17
Maldanidae	—	—	—	13	7	245	2–400
Glycera rouxii	—	—	—	—	1	12	25
Lumbrinereis hibernica	—	—	—	—	—	15	20
Amphitrite cirrata	—	—	—	—	—	10	14
Terebellides stroemii	—	—	—	—	—	12	67
Melinna palmata	—	—	—	—	—	—	787
Lipobranchius jeffreysii	—	—	—	—	—	1	10
Notomastus latericeus	—	—	—	—	—	14	25

TABLE II. DISTRIBUTION OF AMPHIPODS IN KAMES BAY

Station ...	1	2	3	4	5	6	7
Bathyporeia guilliamsoniana	232	60	3	1	—	—	—
Iphinoë trispinosa	3	40	47	30	10	—	—
Siphonoecetes dellavallei	—	—	76	54	79	—	—
Ampelisca brevicornis	—	—	2	13	9	3	2
A. tenuicornis	—	—	—	—	—	8	24

Mollusca

Tellina tenuis is the dominant lamellibranch of the intertidal zone of Kames Bay (Watkin, 1942); it extends a short way into the sublittoral zone and is then replaced by *T. fabula* (Table III). In deeper water still *Abra alba* becomes dominant; *A. alba* is both common and widely distributed in the Clyde area at greater depths than those studied here. The samples of *Ensis ensis* are probably unreliable: as a rule, only the tops of shells are taken by the mud bucket, and there is no means of telling how many individuals had retracted below the sampling depth altogether. The carnivorous gasteropod *Philine aperta* is moderately common at all stations below station 2.

TABLE III. DISTRIBUTION OF MOLLUSCS IN KAMES BAY

Station ...	1	2	3	4	5	6	7
Tellina tenuis	1003	45	4	—	—	—	—
T. fabula	15	98	147	159	124	—	—
Spisula subtruncata	12	69	13	4	—	—	—
Donax vittatus	16	29	—	—	—	—	—
Ensis ensis	5	20	8	13	12	—	—
Venus gallina	3	2	20	3	3	18	—
Cultellus pellucidus	—	—	2	6	25	16	4
Abra alba	—	—	—	5	127	2	4
Dosinia lupinus	—	—	1	4	—	19	83
Thyasira flexuosa	—	—	—	—	2	54	11
Nucula nitida	—	—	4	1	1	52	55
Philine aperta	1	2	46	44	15	56	25

TABLE IV. DISTRIBUTION OF ECHINODERMS IN KAMES BAY

Station ...	1	2	3	4	5	6	7
Echinocardium cordatum	7	3	12	6	3	—	—
Astropecten irregularis	—	1	1	1	3	1	—
Ophiura affinis	1	—	2	1	8	—	2
O. albida	—	—	—	1	7	43	50
Amphiura filiformis	—	—	—	—	—	32	276
A. chiajei	—	—	—	3	—	—	37
Asterias rubens	—	—	—	—	—	6	1
Labidoplax thomsoni	—	—	1	7	1	—	—
Cucumaria elongata	—	—	—	—	—	4	1

Echinodermata

The only echinoderm recorded from the intertidal zone of Kames Bay is *Echinocardium cordatum*; it extends some way into the sublittoral zone and is the only echinoid found in these comparatively shallow waters throughout the year. A marked zonation of ophiuroids exists, but only the fringe of it has been touched here. They extend into deeper water and from the trawl samples taken (*vide infra*) it is evident that the Robertson mud bucket is not ideal for collecting them. *Amphiura filiformis* is at its maximum numbers at station 7 and is replaced in deeper water by *A. chiajei*. *Ophiura affinis* is at a maximum at station 5 and is replaced by *O. albida*. In view of the unsatisfactory nature of the sampling, the figures given in Table IV must be regarded with some suspicion.

The Superficial Fauna

The superficial fauna and the bottom-feeding pelagic animals are not collected by the Robertson bucket. Some of these animals are present in large numbers and must play an important part in the economy of the bottom fauna as a whole. Some are more or less resident. Others, for example, certain pelagic crustaceans are known to migrate inshore from deeper waters at night and at high tide (Watkin, 1941). An intensive and extended investigation would therefore be needed to give a comprehensive picture of their distribution. This has not been attempted here, but a series of trawl samples made at each station in Kames Bay revealed that, at the shallowest stations at least, some of the commonest animals living on or in the substratum had been missed completely by the Robertson bucket (Table V).

TABLE V. ANIMALS TAKEN IN TRAWL SAMPLES AT EACH STATION IN KAMES BAY

	St. 1		St. 2		St. 3		St. 4	St. 5	St. 6	St. 7
	H.W.	L.W.	H.W.	L.W.	H.W.	L.W.	H.W.	H.W.	H.W.	H.W.
Gammarus locusta	588	4566	2	98	2	1	5	5	—	4
Nototropis swammerdammi	877	2730	4	41	—	1	5	2	3	2
Pontocrates arenarius	110	473	—	—	2	1	—	—	—	—
P. norvegicus	31	317	1	—	—	—	—	—	—	—
Pseudocuma cercaria	24	52	2	—	—	—	—	—	—	—
Idotea baltica	136	182	—	—	—	—	—	1	—	—
I. viridis	—	130	—	13	—	—	—	—	—	—
I. granulosa	1	52	—	—	1	—	—	—	—	—
Schistomysis spiritus	89	4	172	107	—	1	—	—	—	—
Crangon vulgaris	21	73	12	9	14	8	1	2	—	—
Praunus flexuosus	6	29	8	14	—	1	—	—	—	—
Platynereis dumerillii	—	—	—	—	—	—	—	1	—	530
Asterias rubens	—	—	—	—	—	—	—	—	1	59
Ophiocomina nigra	—	—	—	—	—	—	—	—	—	60

Crangon vulgaris, *Schistomysis spiritus*, *Idotea baltica*, *Gammarus locusta*, *Nototropis swammerdammi* and *Pseudocuma cercaria* are all plentiful at inshore stations though they are not represented at all in the mud-bucket samples. On the deeper stations the results of the trawl sampling do not alter materially the conclusions already drawn about the relative importance of the various members of the fauna, with two exceptions: (1) *Ophiocomina nigra*, which did not figure in the mud-bucket samples, is now seen to be present at station 7. (2) Contrary to the mud-bucket findings (Table I), *Platynereis dumerillii* is probably more numerous at station 7 than station 6, because of its association with matted vegetable material in the surface mud. The latter is about 50 times more plentiful at station 7. For some reason this material is not normally taken by the bucket. And it is significant that of the total thirty-seven *P. dumerillii* from station 6 (Table I), thirty-one were in the sole bucket sample (from this or any other station) containing an appreciable quantity of the said material.

FAUNA OF WHITE BAY AND A COMPARISON BETWEEN THE TWO BAYS

A similar series of mud-bucket samples was taken in White Bay on seven stations at the same depths as those of Kames Bay (see Fig. 2). The intertidal fauna of White Bay has never been studied. The sublittoral fauna is broadly the same as that of Kames Bay with some interesting differences, doubtless related to the greater exposure and consequent coarser substratum together with the absence of algal debris in White Bay.

Polychaeta

The only remarkable difference between the two bays is the virtual absence of *Platynereis dumerillii*. As we have already noted, this species is commonly associated with matted plant debris so that its absence is only to be expected. The other species have much the same distribution as in Kames Bay though the total numbers are somewhat smaller (see Table VI).

TABLE VI. DISTRIBUTION OF POLYCHAETES IN WHITE BAY

Station ...	1	2	3	4	5	6	7
Nephthys hombergii	1	28	20	24	14	33	26
Spio filicornis	2	11	—	5	1	—	—
Phyllodoce maculata	18	19	2	—	—	—	—
Sigalion mathildae	—	—	8	3	5	—	—
Owenia fusiformis	—	3	27	94	24	123	121
Glycera rouxii	—	—	3	3	7	31	—
Goniada maculata	—	—	2	2	—	50	65
Amphitrite cirrata	—	—	—	—	—	19	12
Lumbrinereis hibernica	—	—	—	1	—	36	35
Scalibregma inflatum	—	—	—	—	—	37	38
Maldanidae	—	—	6	—	18	75	75
Melinna palmata	—	—	—	1	—	282	569
Notomastus latericeus	—	—	—	—	—	12	38

Amphipoda

Some interesting differences appear in the crustacean fauna of White Bay (Table VII). *Bathyporeia guilliamsoniana* is much less common than in Kames Bay, but it appears in much the same part of the beach. *Iphinoë trispinosa* and *Siphonocoetes dellavallei*, both common in Kames Bay, are absent from White Bay. On the other hand, *Ampelisca typica*, which was not found in Kames Bay, is here present in moderate numbers having much the same range as *A. brevicornis*. These differences may be attributable either to the different nature of the substratum or to the lack of vegetable debris in White Bay.

Mollusca

Spisula subtruncata, *Donax vittatus*, *Philine aperta* and *Nucula nitida* are all common in Kames Bay, but are absent or virtually absent from White Bay. *Abra alba*, *Cultellus pellucidus* and *Dosinia lupinus* are present, but in reduced numbers. *Natica alderi*, which is absent from Kames Bay, is found in White Bay where it almost wholly replaces *Philine aperta* as the important carnivorous gasteropod. Species common to the two bays occupy the same position on the beach (see Table VIII).

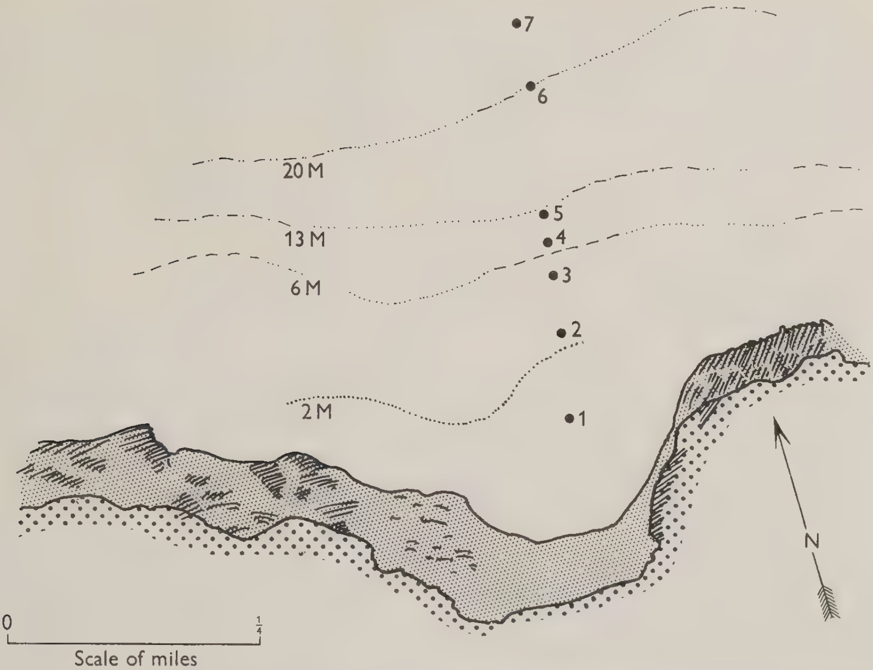

Fig. 2. White Bay, Isle of Cumbrae, showing the position of the stations at which samples were taken.

TABLE VII. DISTRIBUTION OF AMPHIPODS IN WHITE BAY

Station ...	1	2	3	4	5	6	7
Bathyporeia guilliamsoniana	11	35	7	—	—	—	—
Ampelisca brevicornis	—	23	27	7	21	—	—
A. typica	—	—	17	13	5	—	—
A. tenuicornis	—	—	—	3	2	6	3

TABLE VIII. DISTRIBUTION OF MOLLUSCS IN WHITE BAY

Station ...	1	2	3	4	5	6	7
Tellina tenuis	826	1169	47	2	3	—	—
T. fabula	—	83	358	216	44	—	—
Venus gallina	28	19	3	10	9	—	—
Ensis ensis	10	40	12	8	—	—	—
Abra alba	—	—	7	1	4	—	1
Cultellus pellucidus	—	1	8	2	2	2	1
Dosinia lupinus	2	5	11	3	1	11	6
Natica alderi	7	12	8	10	4	4	2
Thyasira flexuosa	—	—	—	5	19	4	—

Echinodermata

Only four species of echinoderm are at all plentiful in Robertson bucket samples though it is quite possible that other species are present and that some of those recorded are more numerous than Table IX indicates. The reduced numbers of holothurians in White Bay is possibly due to the coarser nature of the substratum.

TABLE IX. DISTRIBUTION OF ECHINODERMS IN WHITE BAY

Station ...	1	2	3	4	5	6	7
Echinocardium cordatum	5	22	11	5	—	—	—
Ophiura albida	—	2	10	3	4	3	1
Amphiura chiajei	—	—	—	—	—	—	39
Asterias rubens	—	—	8	5	9	3	10

Thus the zonation of the most abundant animals in the two bays is much the same. The main difference between the bays is that Kames Bay has a more varied and more numerous[1] fauna, particularly at the deeper stations as Tables X–XII clearly show. Presumably this is because of the more sheltered conditions in Kames Bay which result in a finer deposit with large amounts of vegetable debris. There is only one example of an ecological niche being filled by a different animal in the two bays, that of *Philine aperta* in Kames Bay being very largely taken by *Natica alderi* in White Bay. A full list of species found in the two bays is given in the Appendix (pp. 178–180).

TABLE X. NUMBERS OF SPECIES (S) AND INDIVIDUALS (I) FOUND IN TEN SAMPLES AT EACH STATION IN KAMES BAY

Station ...	1		2		3		4		5		6		7	
	S	I	S	I	S	I	S	I	S	I	S	I	S	I
Polychaeta	7	187	3	109	8	93	13	208	13	195	32	1787	31	1370
Amphipoda	8	246	5	74	10	125	10	108	10	120	6	24	3	29
Mollusca	8	1006	9	232	11	218	11	219	11	303	20	230	13	185
Echinodermata	2	8	2	3	4	16	6	19	6	23	7	81	7	368
Totals*	26	1483	19	418	35	455	45	559	42	645	70	2200	57	2026

* Totals include animals not in the four main groups listed above.

TABLE XI. NUMBERS OF SPECIES (S) AND INDIVIDUALS (I) FOUND IN TEN SAMPLES AT EACH STATION IN WHITE BAY

Station ...	1		2		3		4		5		6		7	
	S	I	S	I	S	I	S	I	S	I	S	I	S	I
Polychaeta	11	39	10	73	17	88	18	168	13	95	27	766	21	1043
Amphipoda	7	33	6	63	4	53	3	23	4	29	4	12	1	3
Mollusca	8	878	11	1340	15	488	10	257	9	87	10	37	8	19
Echinodermata	1	5	3	26	6	47	6	19	3	15	6	17	4	51
Totals*	32	965	31	1503	42	676	38	472	30	227	48	833	38	1146

* Totals include animals not in the four main groups listed above.

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[1] But see p. 162, regarding relative numbers of individuals of some species in the two bays.

TABLE XII. COMPARISON OF THE FAUNA OF KAMES BAY AND WHITE BAY

	Kames Bay		White Bay	
	Species	Individuals	Species	Individuals
Polychaeta	47	3949	44	2272
Amphipoda	26	726	14	215
Mollusca	31	2393	24	2108
Echinodermata	12	518	10	180
Totals*	125	7786	102	5822

* The total figures include a small number of animals not belonging to the four main groups.

There are practically no data on other bays with which to compare the present findings. A certain amount of collecting has been carried out in Ettrick Bay on the west side of Bute; the bay resembles Kames Bay more nearly than White Bay, but no sampling in shallow water has been possible there. So far as they go, the results from Ettrick Bay bear out the general tendencies found in Kames and White Bays.

DISTRIBUTION OF ANIMALS ON THE SEA BED

On examining samples from sublittoral deposits it is at once obvious that the density of some animals varies widely in successive sample units from the same station, while other animals appear to be more uniformly distributed. It is possible that the method of sampling might give a spurious impression of the distribution of some species. For instance, the mud bucket might bite deeper on one occasion than on another. But all methods of sampling the sublittoral fauna in use at present have this defect. Judging from the uniform performance of the Robertson bucket at depths where it is visible, there is strong reason for thinking that ten buckets would not vary much in character among themselves at any one station. A more important consideration is that samples of ten units are too small for certain types of statistical treatment.

In considering the nature of the distribution of organisms over an area, Fisher's 'coefficient of dispersion' may be used. It was introduced first in plant ecology, e.g. by Clapham (1936) and Blackman (1942), and later in terrestrial and marine ecology, e.g. by Salt & Hollick (1946), Holme (1950) and Barnes & Marshall (1951). The coefficient of dispersion is given by

$$\Sigma(x-\bar{x})^2/\bar{x}(n-1),$$

where $\Sigma\,(x-\bar{x})^2$ is the sum of squares of the deviations of individual units (x) from the mean ($\bar{x}$) of all the units (n) comprising the sample. The coefficient leads to unity when the population is randomly distributed, is less than one if the population is over-dispersed (i.e. more or less evenly distributed) and greater than one if it is underdispersed (i.e. more or less aggregated). The significance of the departure from unity is tested by

$$1 \pm 2 \times \sqrt{[2n/(n-1)^2]},$$

where, again, n is the number of units in the sample. In the present bucket samples, $n=10$ and the limits of the coefficient for random distribution are therefore 1·9938 and 0·0062. A coefficient greater than 1·9938 may then be taken as significant evidence of aggregation. But obviously, with the lower limit at 0·0062 the coefficient can not be expected to distinguish between random and over-dispersed distribution in samples of 10 units. The sample size need only have been doubled to permit the distinction, although, of course, the larger the sample the better, as the following tabulation shows:

Sample size (n)	Limits of the coefficient of dispersion for random distribution
10	0·0062–1·9938
20	0·3342–1·6658
50	0·5918–1·4082
100	0·7142–1·2858

Departure from randomness may of course be tested by fitting a Poisson distribution from the sample mean and then applying the χ^2 test with $n-2$ degrees of freedom. And Blackman warns that 'when the coefficient of dispersion value is not significantly different from unity the χ^2 test should still be applied since the coefficient of dispersion test, although sensitive as regards aggregation, will not detect certain types of skew distribution'. He gives a field example (a sample of 100 units) where the coefficient obtained though greater than unity is not significantly different from it, yet the evidence of the χ^2 test is that the distribution is not random. Thomson (1952) confirms Blackman. Unfortunately, with a sample size of only 10 units, the direct calculation of χ^2 for a fitted Poisson provides little or no useful information.

Some writers have pointed out that the quantity

$$\Sigma(x-\bar{x})^2/\bar{x},$$

known as the 'index of dispersion', is approximately distributed as χ^2 with $n-1$ degrees of freedom if the data come from a Poisson population. 'For $\bar{x} \geqslant 5$ the χ^2 approximation...is highly satisfactory even for n, the number of observations, as small as 5, and is fairly accurate for $\bar{x} < 5$, although it will tend to give too few significant results' (Bateman, 1950). With $n=10$, the $P=0{\cdot}05$ value of χ^2 is 16·919. And since the index of dispersion is $(n-1)$ times the coefficient of dispersion, this χ^2 value is equivalent to a coefficient of $16{\cdot}919/9=1{\cdot}8799$ which is practically the same as the limit 1·9938 given above. But while a coefficient $> 1{\cdot}9938$ is significant evidence of aggregation, an index $\equiv \chi^2 > 16{\cdot}919$ is merely significant evidence of non-randomness, i.e. the index apparently can not distinguish between aggregation and over-dispersion.

Three other measures of dispersion are discussed by Thomson (1952) but none are appropriate for the present data. We must therefore confine ourselves to employing the coefficient of dispersion alone in an attempt to distinguish between aggregated and non-aggregated species, bearing in mind that animals falling into the latter group may be either randomly distributed, or distributed in a skew but non-Poisson fashion, or overdispersed.

At each station the coefficient of dispersion has been calculated for each species occurring in the ten buckets. Altogether, 459 coefficients are available from the two bays and they range from 0·2593 up to 69·99. Since only 19·4% of coefficients are above the significance limit 1·9938, it seems that non-aggregated distribution (probably chiefly random) is the general rule in the community dwelling on and in the upper layer of the sea bed. As will be seen below, this conclusion requires testing with different sizes of bucket (sample unit).

Aggregation is, of course, out of the question if there is only one individual of a species among ten buckets. But with $n = 10$, the coefficient of dispersion is greater than 1·9938 whenever there are two or more individuals in one bucket and none at all in the remaining nine buckets. Hence significant evidence of aggregation can be obtained from densities of 0·2 per bucket and upwards. Table XIII gives the complete list of species showing evidence of aggregation in at least one sample of ten buckets. In general, the samples of these species do not show consistent evidence of aggregation until the mean per bucket is greater than about 10·0. Above a mean of 10·0, 94·1% of samples have a coefficient indicating aggregation. Below that mean level, the percentage dwindles in a smooth curve until at means of 0·2–0·9 per bucket it is only 26%. This is to be expected from the well-known empirical finding that the smaller the mean for a particular sample unit, the more nearly will a contagious or aggregated distribution conform to the Poisson Law: variance = mean. An obvious corollary is that the demonstration of aggregation will depend to some extent on the dimensions of the sample unit. Thus evidence of aggregation may disappear altogether if a smaller unit (e.g. bucket or quadrat) is used, simply because the mean per unit is reduced. Evans (1952) confirmed this by experimenting (on paper) with different unit (quadrat) sizes on the mapped data from a field in which the plants had been 'completely enumerated'. And he makes the sound point that dispersion should be investigated with more than one size of sample unit.

Partly from the nature of the data and partly from the sketchiness of biological and ecological knowledge at the present time, there is little of value to be concluded from the data on individual species showing some evidence of aggregation (Table XIII) or none at all (Table XIV).

Polychaeta. Ten errant and nine sedentary species of Polychaeta show some evidence of aggregation while six errant and fourteen sedentary species do not. That is 62·5% of errant species but only 39·1% of sedentary species show evidence of aggregation in the two bays. This difference of proportion is significant, $\chi^2 = 4{\cdot}976$, $P = 0{\cdot}03$. Hence errant polychaetes tend more to be aggregated than sedentary polychaetes.

TABLE XIII. SPECIES SHOWING STATISTICAL EVIDENCE OF AGGREGATION IN AT LEAST ONE SAMPLE*

(Samples classified as aggregated (A, i.e. coefficient of dispersion > 1·9938) and non-aggregated (non-A, i.e. coefficient of dispersion < 1·9938) at different mean levels greater than 0·1 individual per bucket.)

	Mean individuals per bucket									
	0·2–0·9		1·0–1·9		2·0–3·9		4·0–9·9		> 10·0	
	A	Non-A	A	Non-A	A	Non-A	A	Non-A	A	Non-A
POLYCHAETA										
Aphrodite aculeata	1	1	—	—	—	—	—	—	—	—
Sigalion mathildae	2	1	—	—	1	2	0	1	—	—
Phyllodoce maculata	1	0	0	2	1	0	—	—	—	—
Eteone longa	2	1	—	—	—	—	—	—	—	—
Platynereis dumerilii	1	1	—	—	1	0	—	—	—	—
Nephthys caeca	1	1	—	—	—	—	—	—	—	—
N. hombergii	—	—	0	2	1	5	1	3	—	—
Glycera rouxii	0	3	2	0	0	2	—	—	—	—
Goniada maculata	0	4	—	—	—	—	3	1	—	—
Lumbrinereis hibernica	—	—	1	0	0	3	—	—	—	—
Spio filicornis	0	2	0	1	—	—	1	0	—	—
Poecilochaetus serpens	0	1	1	0	—	—	—	—	—	—
Scalibregma inflatum	—	—	0	2	1	0	1	0	—	—
Notomastus latericeus	—	—	1	1	1	1	—	—	—	—
Owenia fusiformis	0	2	—	—	1	1	3	1	2	1
Ampharete grubei	1	0	—	—	—	—	—	—	—	—
Melinna palmata	—	—	—	—	—	—	—	—	4	0
Amphitrite cirrata	0	1	2	1	—	—	—	—	—	—
Terebellides stroemii	1	1	0	1	—	—	0	1	—	—
CRUSTACEA										
Ampelisca brevicornis	0	2	0	1	1	2	—	—	—	—
Bathyporeia guilliamsoniana	1	1	0	1	0	1	1	0	1	0
Gammarus locusta	1	2	—	—	—	—	—	—	—	—
Aora typica	1	0	—	—	—	—	—	—	—	—
Siphonoecetes dellavallei	—	—	—	—	—	—	3	0	—	—
Caprella acanthifera	1	0	—	—	—	—	—	—	—	—
Portunus puber	1	1	—	—	—	—	—	—	—	—
MOLLUSCA										
Natica alderi	0	7	1	0	—	—	—	—	—	—
Philine aperta	1	2	0	1	0	1	0	3	—	—
Elysia viridis	1	0	—	—	—	—	—	—	—	—
Lacuna vincta (*Nucula turgida*)	1	0	—	—	—	—	—	—	—	—
Nucula nitida	1	0	—	—	—	—	0	2	—	—
Thyasira flexuosa	0	2	1	0	—	—	1	0	—	—
Dosinia lupinus	0	5	1	2	—	—	0	1	—	—
Venus gallina	0	6	0	4	1	0	—	—	—	—
Donax vittatus	—	—	0	1	1	0	—	—	—	—
Tellina tenuis	1	2	—	—	1	0	1	0	3	0
T. fabula	—	—	0	1	—	—	3	0	4	0
Abra alba	1	4	—	—	—	—	—	—	1	0
Cultellus pellucidus	0	6	1	0	1	0	—	—	—	—
Thracia sp.	2	1	—	—	—	—	—	—	—	—
ECHINODERMATA										
Asterias rubens	1	2	—	—	—	—	—	—	—	—
Amphiura chiajei	0	1	—	—	2	0	—	—	—	—
A. filiformis	1	0	—	—	0	1	—	—	1	0
Ophiura albida	1	4	0	1	—	—	2	0	—	—
O. affinis	1	2	—	—	—	—	—	—	—	—
Echinocardium cordatum	0	6	1	1	0	1	—	—	—	—
Cucumaria elongata	1	1	—	—	—	—	—	—	—	—
Labidoplax thomsoni	1	0	—	—	—	—	—	—	—	—
Totals of samples	29	76	12	23	14	20	20	13	16	1
Percentage of samples aggregated	27·6		34·3		41·2		60·6		94·1	

* A sample is 10 buckets from any one station. The *sample* must not be confused with the *sample unit* which is 1 bucket.

TABLE XIV. SPECIES SHOWING NO SIGN OF AGGREGATION IN ANY SAMPLE WITH MEAN GREATER THAN 0·1 INDIVIDUAL PER BUCKET

	No. of samples of 10 buckets	Range of means
TURBELLARIA		
Cryptocelis alba	4	0·2–1·0
POLYCHAETA		
Gattyana cirrosa	3	0·4–0·8
Harmothoë lunulata	1	0·4
Sthenelais limicola	4	0·2–2·0
Phyllodoce kosteriensis	1	0·3
Eulalia sanguinea	1	0·7
Nephthys longosetosa	3	0·2–2·8
Scoloplos armiger	4	0·2–0·4
Laonice cirrata	2	0·2, 0·3
Spiophanes bombyx	1	0·3
Pygospio elegans	2	0·2
Magelona cincta	2	0·4, 0·5
Cirratulus filiformis	3	0·2–0·9
Stylarioides plumosa	3	0·3–1·5
Lipobranchius jeffreysii	1	0·2
Ammotrypane aulogaster	2	0·2
Pectinaria auricoma	3	0·2–0·6
Lanice conchilega	1	0·3
Pista cristata	1	0·5
Trichobranchius glacialis	4	0·4–1·3
Euchone rosea	1	0·2
CRUSTACEA		
Iphinoë trispinosa	5	0·3–4·4
Diastylis laevis	1	0·2
Hippomedon denticulatus	2	0·7, 0·8
Tryphosites longipes	1	0·3
Ampelisca tenuicornis	6	0·2–2·4
A. typica	3	0·5–1·7
Bathyporeia elegans	2	0·2, 0·3
Haustorius arenarius	1	0·6
Urothoë marina	1	0·3
U. brevicornis	1	0·2
Phoxocephalus holbölli	1	0·6
Pontocrates arenarius	2	0·7, 1·0
Nototropis guttatus	1	0·2
Maera othonis	1	0·4
Dexamine spinosa	1	0·4
Jassa ocia	1	0·5
Eupagurus bernhardus	1	0·3
MOLLUSCA		
Chiton sp.	2	0·3, 0·6
Aporrhais pes-pelecani	3	0·3–0·5
Nassarius reticulatus	1	0·2
Haedropleura septangularis	1	0·4
Nucula tenuis	4	0·2–0·6
Nuculana minuta	1	0·3
Kellia suborbicularis	2	0·3, 0·6
Montacuta ferruginosa	3	0·2–0·5
Venerupis rhomboides	1	0·2
Gari fervensis	1	0·3
Ensis ensis	9	0·5–4·0
*Spisula subtruncata**	5	0·2–5·5
Mya sp.	1	0·7
Corbula gibba	2	0·2, 0·5
ECHINODERMATA		
Astropecten irregularis	1	0·3
Acrocnida brachiata	1	0·2
Holothurian sp.	2	0·4, 1·5

* At the second highest mean, 1·0, *Spisula* was not aggregated (1·7778) but at the highest mean, 5·5, it was very nearly to be regarded so (1·9900)

Crustacea. The writers see nothing to add to what the tables say.

Mollusca. According to Holme (1950), at lower densities *Tellina tenuis* is over-dispersed rather than randomly distributed or aggregated. This is not the case in the present work since samples with means of 0·2, 0·2 and 0·3 per bucket gave coefficients of dispersion equal to 2·0000, 0·8889 and 1·5185 respectively; and samples with means greater than 2·0 per bucket invariably had a coefficient of dispersion denoting aggregation. The disagreement with Holme is probably explained by sample size.

Echinodermata. It is well known that Ophiuroids tend to aggregate, and the present results, with two species of *Ophiura* and two of *Amphiura*, confirm this.

In some cases large local variations in population density may reflect the effects of local differences in the nature of the substratum on the settlement of larvae or their survival after settlement. For this to be the main cause of the marked patchiness of distribution shown by species in Table XIII it must be assumed that the sea bed is a veritable mosaic in which one square yard of the bottom may differ markedly from the next. This is hardly credible. One imagines uniform conditions existing on a sandy or muddy bottom, and certainly the bucket samples suggest uniformity of conditions within each station. The larger variance/mean ratio (coefficient of dispersion) of the species in Table XIII arises more likely from living habits which lead to aggregation of individuals.

Without knowing more about their life histories and habits, it is difficult or impossible to say what might cause aggregation in the individual species listed in Table XIII. Species lacking a dispersal phase and which are fairly sedentary in adult life would naturally tend to collect into family aggregations and one consequence of this in the speciation of polychaetes has already been suggested (Clark, 1952). However, this can not explain aggregations in two of the amphipod species which are active swimmers, leave the sand for breeding and appear in the plankton at night (Watkin, 1941). In these cases the formation of aggregations must be an active process, as it may also be in the case of the polychaete *Goniada maculata* which has a pelagic larva and which is probably an active swimmer in the adult stage. Again, none of the molluscs in Table XIII is known to have lost its dispersive pelagic larval phase; unless differential mortality takes place after the molluscan larvae have settled on the substratum, active aggregation must be assumed. No doubt the factors leading to the formation of aggregations differ not only from group to group but also from species to species. A well-known instance of active aggregation occurs among the Ophiuroidea. Allee (1927) has shown that ophiuroids disperse when living among *Zostera* and can be made to disperse in the laboratory when provided with artificial vegetation in the form of glass rods. Yet living (as they commonly do) on a fairly bare substratum, they form aggregations unless, apparently, the density is too low. Such aggregations on the sea bed have been photographed by Vevers (1951, 1952).

This work was carried out at the Marine Station, Millport. The authors are indebted to Dr R. B. Pike for discussions and information relating to the faunistic work. One of the authors (A. M.) worked on a Carnegie Scholarship 1938–39, the other (R. B. C.) was aided by a grant from The Browne Research Fund of The Royal Society during the summer of 1950.

SUMMARY

A preliminary study has been made of the composition and distribution of the macrofauna living on and in the substratum from low tide level to a depth of about 30 m in Kames Bay and White Bay on the Isle of Cumbrae in the Firth of Clyde. Samples were taken mainly by means of the Robertson mud bucket. A small amount of trawling was done as a check on results for animals dwelling at the surface of the substratum.

The main physical differences between the two bays are: (1) Kames Bay is more sheltered and has a finer deposit of sand or mud at each station with fewer stones and shells than White Bay; (2) in Kames Bay, a substantial quantity of vegetable debris is washed backwards and forwards with the tide in the upper part of the sublittoral zone and there is a large amount of decomposing organic material mixed with the superficial mud at a depth of about 30 m; both these circumstances are practically absent in White Bay.

Full lists are given of the macro-species occurring on and in the sea bed from low-water mark out to 30 m in the two bays. White Bay is somewhat poorer in variety of species and density of individuals than Kames Bay. The only other really important difference is that *Philine aperta*, abundant in Kames Bay, is replaced almost wholly in White Bay by *Natica alderi* as the carnivorous gasteropod.

The distribution of the intertidal fauna of Kames Bay, but not of White Bay, has been extensively studied in the past. The present results therefore extend the picture of animal zonation from high-water mark out to 30 m depth in Kames Bay.

When aggregation occurs, the possibility of its demonstration by means of the coefficient of dispersion depends to some extent on the size of the sample unit. With the Robertson Mud Bucket as the unit, the array of coefficients (calculated from all samples of all species occurring) suggests that non-aggregated distribution (probably chiefly random) is the general rule in the bottom community of the sublittoral. Of some 150 species comprising the invertebrate macrofauna, forty-eight showed significant evidence of aggregation in at least one sample; but the evidence is consistent over all available samples in less than half a dozen species.

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APPENDIX

SPECIES TAKEN IN THE ROBERTSON MUD-BUCKET SAMPLES IN KAMES BAY AND WHITE BAY.

(K, recorded from Kames Bay only. W, recorded from White Bay only.)

TURBELLARIA

K *Cryptocelis alba* (Lang)

NEMERTINEA

Lineus spp.

POLYCHAETA

Aphrodite aculeata L.
K *Lepidonotus squamatus* (L.)
Gattyana cirrosa (Pallas)
K *Harmothoë imbricata* (L.)
W *H. longisetis* (Grube)
H. lunulata (Delle Chiaje)
Sigalion mathildae Audouin & Milne-Edwards
Sthenelais limicola (Ehlers)
Phyllodoce maculata (L.)
K *P. kosteriensis* (Malmgren)
Phyllodoce sp.
K *Eulalia sanguinea* (Oersted)
Eulalia sp.
Eteone longa (Fabricius)
K *E. lactea* Claparède
K *Eteone* sp.
K *Nereis longissima* (Johnston)
Platynereis dumerilii (Audouin & Milne-Edwards)
W *Nephthys caeca* Fabricius
N. hombergii Audouin & Milne-Edwards
W *N. longosetosa* Oersted
Glycera rouxii Audouin & Milne-Edwards
Goniada maculata Oersted
Lumbrinereis hibernica McIntosh
Scoloplos armiger (O. F. Müller)
Laonice cirrata (Sars)
Spiophanes bombyx (Claparède)
Spio filicornis (O. F. Müller)
W *Pygospio elegans* Claparède
K *Magelona papillicornis* F. Müller
M. cincta Ehlers
W *Poecilochaetus serpens* Allen
Cirratulus filiformis Keferstein
K *Chaetozone setosa* Malmgren
Stylarioides plumosa (O. F. Müller)
Scalibregma inflatum Rathke
Lipobranchius jeffreysii (McIntosh)
Ammotrypane aulogaster Rathke
Notomastus latericeus Sars
Maldanidae
K *Rhodine* sp.
K *Axiothella* sp.
Owenia fusiformis Delle Chiaje
W *Pectinaria koreni* Malmgren
P. auricoma (Müller)
K *Ampharete grubei* Malmgren
Anobothrus gracilis (Malmgren)
K *Amphicteis gunneri* (Sars)
Melinna palmata Grube
Amphitrite cirrata (O. F. Müller)
W *Lanice conchilega* (Pallas)
Pista cristata (Müller)
Trichobranchius glacialis Malmgren
Terebellides stroemii Sars
W *Euchone rosea* Langerhans

CRUSTACEA

Cumacea
K *Iphinoë trispinosa* (Goodsir)
W *Lamprops fasciata* G. O. Sars
K *Dyastilis laevis* Norman

Isopoda
W *Cirolana* sp.
K *Idotea granulosa* Rathke

Amphipoda
K *Acidostoma laticorne* O. Sars
Hippomedon denticulatus (Bate)
K *Tryphosites longipes* (Bate & Westwood)
Ampelisca brevicornis (A. Costa)
A. tenuicornis Lilljeborg
W *A. typica* (Bate)
K *Ampelisca* sp.
Bathyporeia guilliamsoniana (Bate)
K *B. elegans*
W *Bathyporeia* sp.
W *Haustorius arenarius* (Slabber)
W *Urothoë marina* (Bate)
U. brevicornis Bate
K *U. elegans* Bate
W *Phoxocephalus holbölli* (Krøyer)
W *Leucothoë incisa* D. Robertson
K *Perioculoides longimanus* (Bate & Westwood)
Pontocrates arenarius (Bate)
K *Nototropis guttatus* (A. Costa)
W *N. swammerdammi* (Milne-Edwards)
K *Eusiris longipes* Boeck
K *Melita obtusata* Montagu
W *Meara othonis* (Milne-Edwards)
K *Gammarus locusta* (L.)
K *Dexamine spinosa* (Montagu)
K *Orchestia* sp.
K *Hyale nilsonii* (Rathke)
K *Aora typica* Krøyer
K *Jassa falcata* (Montagu)
K *J. ocia* (Bates)
K *Siphonoecetes dellavallei* Stebbing
K *Caprella acanthifera* Leach

Decapoda
W *Crangon vulgaris* (L.)
Eupagurus bernhardus (L.)
W *Porcellana longicornis* Pennant
K *Ebalia cranchi* Leach
K *Corystes cassivelaunus* (Pennant)
Portunus puber (L.)
K *P. holsatus* Fabricius

MOLLUSCA

Placophora
W *Chiton* sp.

Gastropoda
W *Gibbula cineraria* (L.)
K *Turritella communis* Risso
W *Capulus ungaricus* (L.)
Aporrhais pes-pelicani da Costa
W *Natica alderi* Forbes
K *Nassarius reticulatus* (L.)
K *Haedropleura septangularis* (Montagu)
K *Haedropleura* sp.
K *Cylichna cylindracea* (Pennant)
Philine aperta Ascanius
Neptunea antiqua (L.)

Lamellibranchia
Nucula turgida Leckenby & Marshall
N. tenuis (Montagu)
Nuculana minuta (Müller)
Thyasira flexuosa (Montagu)
K *Kellia suborbicularis* (Montagu)
Montacuta ferruginosa (Montagu)
K *Cardium echinatum* L.
Dosina lupinus (Montagu)
K *Venus casina* L.
V. striatula (da Costa)
W *Venerupis* (*Tapes*) *rhomboides* (Pennant)
K *Donax vittatus* (da Costa)
Tellina tenuis da Costa
T. fabula Gmelin
Abra alba (Wood)
K *Gari fervensis* (Gmelin)
Cultellus pellucidus (Pennant)
Ensis ensis (L.)
Spisula subtruncata (da Costa)
W *Mya* sp.
Corbula gibba (Olivi)
W *Cochlodesma praetenue* (Montagu)
K *Thracia* sp.
K *Cuspidaria cuspidata* Brown.

ECHINODERMATA

Asteroidea
K *Astropecten irregularis* (Pennant)
Asterias rubens L.

Ophiuroidea
Amphiura chiajei Forbes
A. filiformis (O. F. Müller)
Acrocnida brachiata (Montagu)
K *Ophiura texturata* Lamarck
O. albida Forbes
K *O. affinis* Lütken

Echinoidea
W *Echinus esculentus* L.
Echinocardium cordatum (Pennant)

Holothuroidea
Cucumaria elongata Düben & Koren
Labidoplax thomsoni (Herapath)

PISCES

K *Ammodytes tobianus* L.
K *Lepadogaster bimaculatus* (Donovan)

Additional Species taken by Trawl in Kames Bay

(W, species known also to occur in White Bay)

COELENTERATA

Actinea equina L.

POLYCHAETA

Eulalia fucescens St Joseph
Platynereis dumerilii (Audouin & Milne-Edwards)

CRUSTACEA

Cumacea
Pseudocuma longicornis (Bate)

Isopoda
Idotea baltica (Pallas)
I. pelagica Leach
I. viridis (Slabber)
I. emarginata (Fabricius)
I. linearis (Pennant)
I. granulosa Rathke
Jaera marina (Fabricius)

Amphipoda
Orchomene humilis (A. Costa)
Bathyporeia sp.
Pontocrates arenarius (Bate)
P. norvegicus Boeck
Monoculodes sp.
W *Nototropis swammerdammi* (Milne-Edwards)
Megaluropus agilis Hoek
Melita gladiosa Bate(?)
Gammarus locusta (L.)
Microdeutopus sp.
Amphithoë rubicata (Montagu)
Caprella sp.

Mysidacea
Erythrops elegans (G. O. Sars)
Mysidopsis augusta G. O. Sars
M. gibbosa G. O. Sars
Schistomysis spiritus (Norman)
S. ornata (G. O. Sars)
Praunus flexuosus (Müller)
Acanthomysis longicornis (Milne-Edwards)

Decapoda
Pandalus montagui Leach
Pandalina brevirostris (Rathke)
Hippolyte varians Leach
Spirontocaris cranchi (Leach)
S. pusiola (Krøyer)
Leander serratus (Pennant)
W *Crangon vulgaris* (L.)
Philocheras bispinosus (Hailstone & Westwood)
P. trispinosus (Hailstone)
Eupagurus prideauxii Leach
Portunus corrugatus (Pennant)
Macropodia rostrata L.

MOLLUSCA

Gastropoda
W *Gibbula cineraria* L. (?)
Scaphander lignarius (L.)
Aplysia punctata Cuvier
Pleurobranchus membranaceus (Montagu)
Acanthodoris pilosa (Abildgaard)

Lamellibranchia
Chlamys varia (L.)

ECHINODERMATA

Crinoidea
Antedon bifida (Pennant)
A. petasus (Düben & Koren)

Asteroidea
Asterias rubens L.

Ophiuroidea
Ophiothrix fragilis (Abildgaard)
Ophiocomina nigra (Abildgaard)
Ophiopholis aculeata (L.)

Echinoidea
W *Echinus esculentus* L.

PISCES

Raia clavata L.
Nerophis lumbriciformis (Pennant)
Syngnathus acus L.
Centronotus gunnellus (L.)
Callionymus maculatus (Rafinesque)
Gobius minutus Pallas
Pleuronectes limanda L.
Solea lutea (Risso).

ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

THE ANGULAR SCATTERING OF BLUE, GREEN, AND RED LIGHT BY SEA WATER

By **W. R. G. Atkins** and **H. H. Poole**

Sci. Proc. R. Dublin Soc., Vol. 26 (18), 1954, pp. 313–23.

Previous work on the scattering by sea water of light from a tungsten filament, and of this light filtered through a blue filter (*Proc. Roy. Soc.* B, **140**, 1952, pp. 321–38), has been extended to include green and red light, a R.C.A. 1P22 photomultiplier cell being substituted for the R.C.A. 931A, which had not sufficient green or red sensitivity. To avoid fatigue the exposure was as short as possible and the current below 0·2 μA, usually under 0·1 μA. The same apparatus was used, but with a correction for the effect of light internally reflected in the experimental flask. This becomes large for angles of scatter exceeding about 120°, and renders doubtful the rise in scattering within a zone 1° wide previously deduced from measurements at greater angles. Below the minimum, which again occurred near 120°, the results closely resembled the previous ones, the zonal scattering rising with decreasing angle to some maximum at less than 10°, the lower angular limit of measurement. Blue light was more scattered than either green or red, the difference between the latter two being scarcely significant. Successive filtering through collodion filters 1·4, 0·5 and 0·1 μ average pore diameter reduced the scattering by sea water to about the same value as that of the purest doubly distilled water, and increased the difference between blue light and the other colours. H.H.P.

THE EUPHAUSIID CRUSTACEANS OF SOUTHERN AFRICAN WATERS

By **Brian P. Boden**

Trans. Roy. Soc. S. Afr., Vol. 34 (1), 1954, pp. 181–243.

The euphausiid crustaceans from several extensive collections made in the waters around southern Africa have been examined. Forty-two species, belonging to nine genera, are described and fully illustrated. One new species, *Thysanopoda subaequalis*, is described, and sixteen new distribution records are added. Keys to the genera and species are included. A short comment is made on the hydrographic conditions in the area. The distribution of species is treated briefly, with extra emphasis on those species whose more southern representatives have been investigated by other workers. B.P.B.

QUELQUES CARACTÉRISTIQUES BIOLOGIQUES DE *LOPHIUS PISCATORIUS* L.

By **L. Brull** and **Y. Cuypers**

(avec la collaboration de L. Dubois et L. Wilsens)

Arch. int. Physiol., T. 62, 1954, pp. 70–5.

The following properties of *Lophius* blood and urine have been determined: (1) Systemic blood pressure is equal to 40–50 cm of water, with systolic variations of 3–5 cm. (2) There is about 2 g% haemoglobin in the blood. Variations do not depend on the size of the fish. (3) Δ of bladder urine is lower than, or equal to, that of plasma in ten measurements out of twelve, and slightly above in the remaining two. (4) Plasma magnesium (normally 6·5 mg% or less) appears to increase from the time when the fish is caught, rising to 9 or even 16·7 mg. (5) Urine magnesium increases similarly after catching, reaching about 300 mg%. L.B.

SPICULE FORM IN *LEUCOSOLENIA COMPLICATA*

By **W. C. Jones**

Quart. J. micr. Sci., Vol. 95, 1954, pp. 191–203.

The tri- and quadriradiates of *Leucosolenia complicata* are described in four aspects, surface view, transverse view, side view and planar view. The form and size in all four aspects vary with the distance from the oscular edge.

Each spicule ray tends to grow in a plane containing the optic axis. The oscular ray planes intersect at an angle which varies from 160° at the oscular edge to 140° at the base of the tube.

A preliminary discussion is given on some factors controlling spicule form.

W.C.J.

THE MECHANISM OF PROBOSCIS MOVEMENT IN *ARENICOLA*

By **G. P. Wells**

Quart. J. micr. Sci., Vol. 95, 1954, pp. 251–70.

The mechanism of proboscis movement is analysed in detail in *Arenicola marina* L. and *A. ecaudata* Johnston, and discussed in relation to the properties of the hydrostatic skeleton.

Proboscis activity is based on the same three-stage cycle of movements in both species, but they differ anatomically and in their hydrostatic relationships. In *ecaudata*, the forward movement of body-fluid which extrudes and distends the proboscis is largely due to the contraction of the gular membrane and septal pouches. In *marina*, the essential mechanism is the relaxation of the oral region which allows the general coelomic pressure to extrude the proboscis. The gular membrane of *marina* contracts as that of *ecaudata* does, but its anatomy is different and it appears to be a degenerating structure as far as proboscis extrusion is concerned.

The proboscis is used both in feeding and in burrowing; in the latter case nothing enters through the mouth; the difference is largely caused by variation in the timing of withdrawal relative to the 3-stage cycle. G.P.W.

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BIOLOGICALLY ACTIVE COMPOUNDS IN THE SEA

By R. Johnston

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INTRODUCTION

It is becoming recognized that metabolites released into aquatic and other environments may have vital significance for associated communities. For the particular habitat of the sea, Lucas (1938) indicated tentatively some of the possibilities and later (1947, 1949) reviewed some of the extensive evidence which provided support for a new concept of 'non-predatory' inter-relationships. External metabolites ('ectocrines') formed by some organisms were envisaged as favouring or hindering the growth of other members of the community, their effects varying according to their concentrations. Although a few might extend their effects in relatively large concentrations, many (perhaps the most important) were regarded as effective at extreme dilution. Metabolites could conceivably neutralize the growth-inhibiting or growth-promoting effects of other substances, presumably including other metabolites. The production and persistence of metabolites in a body of sea water imposed a 'biological history' on the water and provided a basis for ecological 'succession'. The general evidence of 'succession', like that implying 'animal exclusion' (Hardy, 1935), almost demanded such a concept in support.

The concept of 'non-predatory' relationships drew support from a wide variety of biological observations but, until recently, little biochemical work has been carried out to detect or identify metabolites. The early experiments (e.g. Allen & Nelson, 1910), showing the importance of organic matter for establishing successful cultures, led logically to Harvey's demonstration (1933 and later) of the necessity for accessory factors in the growth of some marine diatoms, while other workers showed the significance of pro- and antibiotic substances in the culture of algae and other micro-organisms (e.g. Lefevre *et al.*, 1952). A few workers (e.g. Harvey, 1939; Matsudiara, 1939) had demonstrated differences in sea waters as culture media, and Hutchinson (1943), for example, produced evidence of a free vitamin (thiamin) in lake water. Recently a number of prominent contributions have been made by the workers at the Haskins Laboratories (New York) who have investigated with great precision the nutrition of a few marine and freshwater micro-organisms. Their work is demonstrating interdependencies such as: '(1) the interchange of growth factors; (2) the lowering of inhibitory concentrations of several major mineral nutrients, especially phosphate; and (3) the preferential utilization of minerals, including trace metals, [which] may condition waters, bringing their concentrations into the optimal zones for succeeding forms'. They consider that the practical aim, 'to predict algal successions and blooms—may be achieved through a comprehensive knowledge of vitamin cycles as well as mineral cycles. An immediate problem is to trace the thiamin and cobalamin cycles' (Provasoli & Pintner, 1953, p. 849).

Meanwhile, Wilson (1932 and later) had observed that some polychaete larvae would only settle on limited types of sand which were almost certainly associated with other and perhaps specific forms of life. In another series of experiments (Wilson, 1951; Wilson & Armstrong, 1952, 1954) he demonstrated that the eggs and larvae of the sea urchin (and two species of marine worms studied less intensively) would grow more satisfactorily in water from the Celtic Sea than in English Channel water. It is significant that these waters can be distinguished by the characteristic species of *Sagitta* so that Wilson's experiments link directly with Russell's demonstration (1935) of the importance of 'western' water for good productivity in the English Channel. The general idea of sea-water characterization by chaetognath indicator species was developed for more extensive sea areas by Fraser (1937, 1939) and some postulates were made with regard to possible operative factors (Fraser, 1952).

Although in a broad sense closely related to their hydrographic environment, it is now familiar knowledge that planktonic communities may sometimes be sharply distinguished even within a water mass which is homogeneous with regard to all the usual hydrographic characteristics, including nutrients. Not only may hydrographic and nutrient contents of natural waters be significant, and inorganic micro-constituents at a more subtle level, but also the organic compounds present as the result of past and present metabolic processes. Indeed, in so far as final productivity depends on the success of photosynthetic micro-organisms, any demonstration of the dependence of these organisms on external metabolites points indirectly, if not directly, to the significance of such metabolites for the rest of the community.

It may even be legitimate to speculate further on the possible value of such work. The sensitivity of some micro-organisms to certain metabolites has been shown to be extremely high, their productivity being entirely dependent on the availability of these metabolites, whatever the grosser nutrients available (see, for example, Hamilton, Hutner & Provasoli, 1952). The possibility arises, therefore, of initiating a chain reaction of biological events ('succession') by adding to natural waters metabolites likely to encourage a desirable sequence and to suppress undesirable types. Since no additional nutrients are involved, and since the quantities of the metabolites may be relatively very small, this possibility may lead to more practical methods of controlling some aspects of aquatic life than have so far been suggested.

Thus the study of biologically active organic substances[1] in the sea is at least indirectly relevant to fishery problems. In time, some compounds may be shown to be directly relevant—perhaps by favouring or hindering the development of fish eggs or larvae, or even affecting the adult life of shellfish and true fish. One hint may be found in the observations of Collier, Ray, Magnitzky & Bell (1953) that the active feeding of oysters is related to the concentration in the sea water of certain organic substances, which respond to the *N*-ethyl carbazole test for carbohydrates. Perhaps another lies in the results of fish-behaviour studies. It has been suggested that salmon may be able to distinguish the products of one organism from another even at great dilutions and perhaps thereby the waters of one stream from others (see, for example, Lissmann's review, 1954). The requirements which must be fulfilled to satisfy the theory that fish discriminate 'home' waters by perception of special characteristic chemical constituents have been described by Hasler & Wisby (1951). They have tracked down the characteristic odour in one instance to a volatile aromatic substance.

PREVIOUS ORGANIC ANALYSES OF SEA WATER

Much of the early work on the analysis of organic matter in the sea relied on combustion or powerful oxidizing agents (Bond, 1933; Krogh & Keys, 1934) to give an estimate of the total carbon present, and gave no clue regarding the nature of the compounds involved. A most extensive review of analyses of marine organisms, including plankton, from the earliest records to 1944 has been prepared by Vinogradov (1953). The analyses are chiefly concerned with moisture content, ash and assays of C, H, N, P and many other elements. Recently, the particulate matter (organic and inorganic) in sea water has been studied and partly analysed by Armstrong & Atkins (1950) and also by Fox, Oppenheimer & Kittredge (1953). The inanimate organic matter in the sea, according to the most recent estimate (Fox *et al.*, 1953), occurs to the extent of approximately 60% as particulate matter and about 40% as dissolved substances.

The separation and analysis of dissolved organic matter isolated from sea water has seldom been reported. Wangersky (1952) isolated by dialysis what was considered to be a rhamnose-containing polysaccharide which affected the feeding of oysters (Collier *et al.*, 1950).

It is perhaps reasonable to consider as potentially soluble some of the organic compounds found internally in marine plants and animals. An up to date and comprehensive review and bibliography has been prepared by Black (1954) of the organic chemical analysis of many of the constituents of brown, red and green algae.

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[1] When the organic matter is isolated from a sample of sea water whose biological history will normally be unknown, it is more accurate to describe any active compounds simply as biologically active (to organism 'X') than to describe them as 'ectocrines' which may imply knowledge of the original sources which is not yet available.

Work at Aberdeen

With such possibilities in mind, work was begun at Aberdeen to see whether natural waters could be distinguished by their organic content and, specifically, whether the organic content could be shown to have variable significance for representative marine micro-organisms. This paper gives a brief outline of the first results. It will have served its purpose if it provokes discussion, perhaps some pooling of ideas, and stimulates further work on the biological and biochemical sides, for assuredly the two must go together.

Possible lines of advance in the study of organic matter in the sea may lie in (*a*) direct organic analysis of extracts from large amounts of sea water, (*b*) intensive assay of natural and enriched sea waters with specific micro-organisms, and (*c*) isolation of metabolites from pure mass cultures. Each of these possibilities might make an important contribution to marine science but the total work required would be very large. A more incisive attack, which might enable important features to be detected relatively quickly, demands a speedy method of isolating organic matter from relatively small samples of filtered sea water, followed by analysis of the extract and biological testing of some of the components. Final details of the method to be adopted are not yet settled, but the following paragraphs describe its evolution and the results to date.

Methods of Isolation

The earliest experiments were straightforward applications of normal methods of isolating organic compounds to the separation of dissolved organic matter from sea water. In each experiment the sea-water sample was first passed through a $1\,\mu$ filter soon after collection to remove detritus, plankton and, as far as possible, all the nanoplankton. Various methods of isolation were tried and the results obtained are summarized below.

(1) *Evaporation and extraction.* Sea water was evaporated at 30–40° C. under reduced pressure and portions of the salt residue extracted with various organic liquids. Most common solvents, e.g. alcohol, acetone, ether and, to a lesser extent, chloroform, dissolved troublesome amounts of salts in addition to any organic matter. Benzene gave least salt contamination but only a very small amount of extract. Handling of the original hygroscopic mass of salts was difficult and complete extraction was laborious.

(2) *Solvent extraction.* Extraction with organic liquids immiscible with sea water was a convenient and rapid method of isolating some of the fatty, waxy and highly coloured substances. Only solvents which did not dissolve appreciable amounts of salts were suitable, such as benzene.

(3) *Dialysis.* Sea water was dialysed against tap water to yield on evaporation organic residues of high molecular weight. The method demands the handling of fairly large volumes of liquid in order to obtain a reasonable yield but is perhaps one of the most useful, although only applicable to a restricted range of organic compounds.

(4) *Ion-exchange treatment.* Direct ion-exchange treatment of sea water proved quite unsatisfactory owing to the high salt concentration. The process can be useful, however, for removing final traces of salts from any type of extract.

(5) *Adsorption.* Many substances can be used to adsorb some part of the organic (and at the same time some inorganic) matter from sea water. Carbon, alumina, cotton-wool and cellulose powder were tried. The method is simple, fairly rapid, and not very selective in the types of organic compounds removed. Some results for carbon extracts are described later.

(6) *Electrodialysis.* The most recent separations were by this method, which in theory should yield the total organic matter more or less unchanged and free from salts. Supplies are becoming available of ion-exchange membranes which are impermeable to organic substances except perhaps the most mobile fatty acid ions, e.g. formate, acetate. Objections to the use of membranes such as parchment or cellophane, which permit the passage out of the solution of, for example, amino-acids and neutral organic compounds of low molecular weight are thus overcome. The method is convenient but slow, and further development of a suitable type of apparatus is required. Multi-membrane types have the particular advantage of minimizing temperature breakdown and electrolytic reduction or oxidation of sensitive organic materials.

Qualitative Analysis of Organic Extracts

Many methods were attempted. In general, direct organic analysis, spot tests, micro-reactions and paper chromatograms sprayed with selective reagents yielded relatively indecisive results on the gross extracts, and also after attempted fractionation, perhaps because separations were not complete. However, several clues were obtained.

Among the most common components of the carbon extracts were (1) an insoluble white substance similar to that described by Wilson & Armstrong (1952, p. 338), (2) coloured material, possibly carotenoid, soluble in benzene, petroleum ethers, etc., (3) a fairly large proportion of brownish waxy or fatty matter, and (4) yellowish orange matter with a bright blue fluorescence in ultra-violet light (probably related to the 'gelbstoff' of Kalle, 1949). A number of other components were detected by paper chromatography and examination in ultra-violet light but could not be satisfactorily separated by preliminary attempts using column chromatography.

Empirical Organic Analysis of Sea Water Samples

Salt-free carbon extracts from filtered samples of sea water are readily compared by preparing paper chromatograms of portions of the extracts (see, for example, Balston & Talbot, 1952, for general technical details).

Sea water (2 l.) is shaken with activated carbon (*c.* 10 g) and allowed to stand. Most of the liquid can then be sucked or siphoned off, the carbon recovered on a filter and washed with distilled water until chloride-free. A cold extraction process, using alcohol followed by an alcohol-benzene mixture, has been used to recover the organic matter from the carbon.

Because of variations in the adsorptive powers of various carbons and in their extractable impurities, it is essential to standardize the technique by using only one type of carbon and by including carbon blanks. These blanks consist of carbon, shaken with sample volumes of 3·5 % saline solution, and washed and extracted in the same way as the sea-water samples.

When chromatograms of the extracts and the carbon blanks were compared by ultra-violet light, different patterns and numbers of spots were seen. Provided interfering inorganic matter has been removed, these spots represent different organic components of the sea-water samples. Unknown proportions of only those compounds which are adsorbed by carbon from sea water are represented in the extract, however, and not necessarily all the soluble organic matter present.

Any suitable combination of adsorbent, solvent or paper chromatogram technique can be tried. The method yields comparative organic chemical analyses of extracts from small sea-water samples with minimum effort. For known constituents it could be developed to give approximate quantitative analysis by colorimetric methods or for bio-assay by plating or culture techniques. Especially in the case of microbiological assay it would be necessary to ensure adequate control measures to eliminate spurious effects arising from impurities in the activated carbon and in the solvents used.

Biological Effects of Some Organic Isolates

Since the preliminary qualitative organic analysis of such extracts had proved unrewarding, it was important to decide whether further attempts were worth while. The immediate value of the study of organic matter must be dependent largely on some components proving to be of biological interest, possessing, for example, growth-promoting or growth-inhibiting properties. It was not possible to test biological activity in a wide range of ways at a number of different concentrations because of the small quantities of each fraction then available, but it was felt that the responses of some common marine phytoplankton species might provide indications of biological activity of possible significance for fisheries.

An artificial basal medium was prepared according to formulae devised by Provasoli & Pintner (1953, p. 842). The plankton cultures were kindly supplied by Dr Mary Parke of the Laboratory at Plymouth. Growth was assessed by measuring change in optical density of small cultures in closed tubes. A list of the eleven types which were grown in sixteen different media is given in Table I. The various additions to the basal inorganic medium are listed in Table II. Usually the organic extract was obtained from sea water by means of adsorption on carbon. In assessing the subsequent growth of cultures, differences in optical density of $\geqslant 8$ units are significant at the 90 % level; $\geqslant 10$ units at the 95 % level.

The algae reacted to several of the fractions, exhibiting both increased and decreased growth, as compared with controls. Further (and perhaps different) instances of these effects would probably have been observed

TABLE I

Culture used	Medium no. (see Table II)																Number of experiments with values		
																	≥10		Others
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	+	−	
Skeletonema costatum (Grev.) Cleve	−12	5	−5	0	7	0	−13	6	3	9	−10	5	−7	−8	7	3	0	3	13
Mixed culture (Aberdeen)*	14	29	1	8	35	−3	6	27	−5	12	−9	21	2	−5	−3	9	6	0	10
Thalassiosira gravida Cleve	3	−3	−11	9	10	12	−1	32	6	10	−6	18	5	2	4	5	5	1	10
Chromulina pusilla Butcher	3	−4	5	3	−5	−22	−13	39	−7	7	6	−2	2	3	−1	15	2	2	12
Chaetoceros decipiens Cleve	5	3	2	2	−3	−5	12	−1	10	−6	−9	−3	9	6	−2	−1	2	0	14
Thalassiothrix frauenfeldii Grun. in Cleve & Grun.	−5	2	12	−4	10	17	−2	30	−8	3	13	11	−8	6	−6	3	6	0	10
Prorocentrum micans Ehrenberg	4	5	−4	1	8	−9	18	24	−10	−10	1	1	−1	7	2	−1	2	2	12
Phaeodactylum tricornutum Bohlin	4	21	10	19	26	7	−9	35	22	10	−16	19	16	−1	30	23	11	1	4
Plymouth No. 103 *Gymnodinium* sp.	3	2	−4	5	11	6	8	36	13	2	38	6	10	−16	−3	5	5	1	10
Plymouth No. 81 *Dunaliella* sp.	51	61	93	96	47	73	57	254	130	57	50	176	65	59	116	68	16	0	0
Isochrysis galbana Parke	0	40	9	−12	13	−4	5	39	−4	15	17	2	4	10	−11	4	6	2	8
																	61	12	103
Successful cultures	2	4	3	2	7	3	3	9	4	5	4	5	3	2	2	3	34·6%	6·8%	58·6%
Unsuccessful cultures	1	0	1	1	0	1	2	0	1	1	2	0	0	1	1	0			
Little change†	8	7	7	8	4	7	6	2	6	5	5	6	8	8	8	8			

Values in the table represent changes in optical density (log $I_0/I \times 10^3$) between 1st (10. viii. 53) and 18th day (28. viii. 53), for 5 ml. cultures in 1 cm diameter tubes. Light filter for Spekker, Ilford 601. Temperature range 15·4–15.7° C. Light intensity *c.* 2200 lux, continuous. Media adjusted to initial pH 8·0.

* *Chaetoceros ceratosporus* (or *gracilis*) and a *Chlorococcus* sp.

† Includes unsuccessful cultures of colourless species.

by testing a range of concentrations. One particular fraction was found to promote greater growth in nine of the eleven species tested. Other fractions affected smaller numbers of species favourably or unfavourably. The algal cultures used, although species-pure, were not bacteria-free, so that the observed changes in optical density of the cultures may not refer directly to the original medium supplied but to a medium modified by bacterial metabolism. Large-scale bacterial proliferation could also directly alter the optical density of the cultures. To what extent such effects complicate the assessment of the biological activity on the algae is not known at present, although examination of the cultures at the end of the experiment showed no obvious signs of bacterial colonies. Many of the cultures persisted for several months after the completion of the experiments. In any event, although of fundamental interest, the distinction is of less immediate importance, since most, if not all, algae normally grow in association with bacteria, from which they may well acquire some accessory nutrients.

TABLE II

Medium no.	Supplement to basal medium (5 ml.)
1	0·2 ml. of a stable alkaline saline earth extract
2	0·2 ml. distilled water
3	0·18 ml. D.W. + 0·02 ml. of the 5 % alcohol extract of carbon adsorbate
4	0·18 ml. D.W. + 0·02 ml. of the 20 % alcohol extract of carbon adsorbate
5	0·18 ml. D.W. + 0·02 ml. of Fraction 3, cellulose powder column chromatogram of carbon adsorbate
6	0·18 ml. D.W. + 0·02 ml. of Fraction 8, cellulose powder column chromatogram of carbon adsorbate
7	0·18 ml. D.W. + 0·02 ml. of Fraction 19, cellulose powder column chromatogram of carbon adsorbate
8	0·18 ml. D.W. + 0·02 ml. of Fraction 23, cellulose powder column chromatogram of carbon adsorbate
9	0·18 ml. D.W. + 0·02 ml. of Fraction 37, cellulose powder column chromatogram of carbon adsorbate
10	0·18 ml. D.W. + 0·02 ml. of Fraction 48, cellulose powder column chromatogram of carbon adsorbate
11	0·18 ml. D.W. + 0·02 ml. of Fraction 52, cellulose powder column chromatogram of carbon adsorbate
12	0·18 ml. D.W. + 0·02 ml. of the aqueous extract of Whatman No. 1 filter paper
13	0·18 ml. D.W. + 0·02 ml. of the second carbon extract of water from 30 m, 58° 35′ N., 00° 00′ April 1953
14	0·18 ml. D.W. + 0·02 ml. of the whole carbon extract, water B 21c, February 1953
15	0·18 ml. D.W. + 0·02 ml. of 18/44 Sutcliffe Speakman and Co., Quality 110, carbon blank
16	0·18 ml. D.W. + 0·02 ml. of the 50 % alcohol extract of carbon adsorbate

It is difficult to relate the concentrations of organic isolates in the culture media to those of the appropriate fractions in the original sea-water samples since so many losses inevitably occur during isolation and fractionation. The *in vitro* concentrations used probably ranged between 2 and 40 times those occurring naturally, but it should be stressed that this is a very approximate reckoning, and these estimates may be too high.

Future Lines of Research

This preliminary work suggests that a method based on (*a*) isolation of organic matter by electrodialysis using ion-exchange membranes, (*b*) separation by paper chromatography, and (*c*) biological testing using a number of organisms, should meet the requirements of sensitivity and selectivity. An examination of the complete organic matter in solution is still reasonably economic in labour.

It is hoped that, in this way, it may be possible to 'label' water samples (and consequently the water masses from which they derive) somewhat grossly according to (1) the number and chromatographic position of some organic compounds, and (2) the nature of some of their biological effects—even before it is possible to indicate the chemical nature of the organic differences concerned. Such results should be of immediate help in the interpretation of the ecological situation, whilst the observed biological differences should give pointers to the sections of the extracts most deserving chemical analyses. It may conceivably be possible to apply the results to fish behaviour and/or development.

Although the main chemical effort will be directed to this end, it is not intended to omit analyses of the dissolved organic matter. Preparations are in hand for continuous de-salting of sea water in fairly large quantities by ion-exchange electrodialysis. Work already done can be utilized to construct a more effective course of preliminary separation, and automatic fractionation apparatus has recently been obtained which will facilitate chromatography. Individual fractions can then be tested for biological activity and facilities are available for ultra-violet and infra-red spectroscopic analysis of interesting components. It may be possible, using this knowledge and experience, to obtain by a shorter method sufficient of the interesting fractions to permit fuller qualitative organic micro-analysis.

SUMMARY

The concept of external metabolites in the sea, which has received support from biological investigations, invites chemical corroboration which, until recently, has not been forthcoming. Separations of organic matter from filtered sea water using a variety of methods are reported and a preliminary assessment made. Organic residues obtained from different open sea sources by adsorption on carbon give rise to different paper chromatogram patterns. Certain organic components of a carbon adsorbate contain substances which affect the growth of a number of marine phytoplankton organisms. Further research is planned employing methods of isolation and chromatography of the dissolved organic matter from different sea waters and the assessment of biological activity of certain fractions by means of common marine organisms. Some aspects of the significance of biologically active compounds in the sea are mentioned.

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UNDERWATER OBSERVATIONS ON ROCKS OFF STOKE POINT AND DARTMOUTH

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The Lower Limit of *LAMINARIA* and other Algae

In the vicinity of the Stoke Point rocks which lie ¼ mile offshore, plants of *Laminaria*—*L. hyperborea* (Gunn.) Fosl.—disappear below approximately 17 m, from low-water spring tide level. This limit, described in the preliminary note (Forster, 1954, this *Journal*, Vol. 33, pp. 341–4) has been confirmed during five dives in the same area in August 1954. The lower limit of *Dictyopteris membranacea* (Stackh.) Bate, which largely replaces *Laminaria*, has not yet been fully determined, but in fairly broad gulleys it fades out at 25 m and is followed by purely animal growths. Twenty-five metres is, therefore, probably close to its lower limit for this particular area.

In contrast, at the East Blackstone which is ½ mile offshore near Dartmouth, the *Laminaria* only penetrated to 5–6 m. It was followed, down to 8 m, by scattered plants of *Dictyota dichotoma* (Huds.) Lamour., and very sparse red algae chiefly *Heterosiphonia plumosa* (Ellis) Batt. Closer inshore, and in Torbay, *Saccorhiza polyschides* (Lightf.) Batt. apparently replaced the *Laminaria hyperborea*. The lower limit of *Saccorhiza* was not observed, but at two separate positions it became very sparse by 6 m. These observations were made during nine dives in September 1954.

The Sessile Fauna

The results obtained in 1953 from Stoke Point rocks have been confirmed during 1954, and further dives made to slightly greater depths. From the end of the *Laminaria* zone at 17 m down to 25 m, the rocks are often fairly flat with a series of widely separate ridges about 2 m high, running roughly north and south. The sea bottom, viewed from 3 or 4 m away, appeared as a dark brown field of *Dictyopteris* relieved by large yellow clumps of *Cliona celata* Grant (non-boring form) and pink sea-fans, *Eunicella verrucosa* (Pallas). There were roughly two *Cliona* and three *Eunicella* colonies per 10 sq.m.

Beyond 25 m the rocks were sometimes irregular, forming gulleys and pockets with a bottom of coarse sand. The gulley walls from 25 to 28 m, though not steeply sloping, were devoid of algae. Here the fauna was composed chiefly of Coelenterata and Bryozoa. *Corynactis viridis* Allman, though still common, was not as abundant as it was on vertical gulley walls at 18–20 m, when the algae were also excluded. Both *Alcyonium glomeratum* (Hassall) and

Epizoanthus wrightii (H. and S.) also occurred frequently, the latter with numerous bright orange and yellow polyps forming patches of up to approximately 1 sq.m in area. A few colonies of *Sertularella gayi* (Lamouroux) appeared for the first time, this species being common on the Eddystone trawling grounds in 45–65 m. The chief Bryozoa were erect forms, *Cellaria fistulosa* (L.), *Flustra papyracea* (Ellis & Solander), with scattered colonies of *Lepralia foliacea* (E. & S.).

The sessile rock fauna at the E. Blackstone was considerably different from that of Stoke Point rocks. From 4·5 to 6 m, very approximately, on vertical or overhanging rock faces small mussels (*Mytilus* sp.) were abundant. The small white anemone *Actinothoë sphyrodeta* (Gosse) occurred commonly both with the *Mytilus* and in patches on its own.

From 6 m down to 18 m, which was the lower limit reached, the following species were very common: *Nemertesia* (*Antennularia*) *antennina* (L.), *Tubularia indivisa* L., *Sertularella gayi* (hydroids); *Scrupocellaria scruposa* (E. & S.), *Cellaria fistulosa*, *Flustra papyracea* (Bryozoa); and *Antedon bifida* (Pennant). No zonation of these species with depths was observed; the rock surface being covered over considerable areas either by the hydroids, the Bryozoa, or *Antedon* or combinations of these groups especially hydroids and Bryozoa. There were also small patches of *Actinothoë*, groups of *Metridium senile* (L.), numerous *Caryophyllia—C. smithi* Stokes, cup coral—and the ubiquitous *Corynactis*, though only scattered polyps. Only two *Eunicella* and one *Axinella—A. polyploides* (Schmidt)—were seen and may, therefore, be taken as rare.

One dive was made at the E. Cod Rock near Berry Head. In contrast to the igneous rock of E. Blackstone, this rock was composed of limestone. At a depth of 6–7 m the rock surface was honeycombed by the boring mollusc *Hiatella arctica* (L.). Besides *Antedon*, *Alcyonium digitatum* L., and *Mytilus* sp., which were common, there were also many anemones usually inhabiting *Hiatella* burrows. The anemones were *Tealia felina* (L.) and *Cereus pedunculatus* (Pennant). *Metridium* was present in smaller numbers and growing normally on the rock.

DISCUSSION

During the diving the water was found to be much more turbid between Dartmouth and Torbay than near Stoke Point, where during a calm spell of weather the bottom can be seen clearly at a depth of 11 m, whereas in Torbay even during a prolonged period of offshore winds the comparable limit was only 2–3 m. The shallow penetration of the algae at the E. Blackstone and nearby is clearly the result of growing in more continually turbid water. With the fauna, however, only *Sertularella*, *Flustra* and *Cellaria* were common to both areas. The contrast in the fauna between the two areas was not simply an alteration in the life zones of various species, but the species themselves were mostly different. Much of the E. Blackstone fauna was not unfamiliar, since *Nemertesia* (Antennularia), *Actinothoë*, *Mytilus* sp. and *Antedon* are rather common species in Plymouth Sound. Both the Sound and the Dartmouth–Torbay area are sheltered from the prevailing west and south-west winds, and thus from the more violent wave action; but they also differ from the Stoke Point area in the greater turbidity of the water. Thus the change in the sessile fauna may not be solely related to wave action.

It was surprising to find the sea so much more turbid in the sheltered Dartmouth–Torbay area than in the exposed Stoke Point area, as the reverse had been expected. Although this situation might possibly be attributed merely to the sea-bed being largely mud in Torbay but sand beyond the rocks at Stoke Point, it seems more reasonable to assume that the turbidity does not arise *in situ* but results from a continual replacement of water containing suspended material. For Torbay itself has extremely weak tidal streams and is very well sheltered from wave action except from the east. In September 1954, when the observations were made, there had been no strong east wind for at least two months; so unless the water was being replaced one would expect it to have been much clearer than at Stoke Point where any south-westerly gale turns the sea cloudy for several days. Dr L. H. N. Cooper suggests that the turbidity may originate in the violent water mixing which takes place off Portland Bill, 40 miles to the eastward.

Acknowledgement

I am much indebted to Mr R. U. Gooding for his help and assistance both under and above water; to Messrs Siebe Gorman and Co. who were kind enough to loan Mr Gooding apparatus; and to the Royal Society for a grant with which much of my own apparatus was obtained.

Summary

A description is given of the commonest sublittoral algae and sessile animals found on rock at Stoke Point Reef and at the E. Blackstone rock near Dartmouth to a maximum depth of 28 m. The E. Blackstone fauna resembled that of Plymouth Sound. The marked change which has been found is ascribed to the greater turbidity of the water near Dartmouth and the increased shelter from wave action.

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OBSERVATIONS ON THE CILIARY CURRENTS OF THE JELLY-FISH *AURELIA AURITA* L.

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(Text-figs. 1–7)

The common jelly-fish *Aurelia aurita* has been a popular species for the study of medusan physiology, for its abundance, transparency and docility make it easy to observe. Unfortunately, studies of the animal as a whole are rare, many investigators having confined themselves to isolated parts or functions, and it is sometimes difficult to combine the accounts in the literature. Such a difficulty arose on attempting to assess the role of ciliary currents in feeding. Widmark (1913) described only the internal currents, Orton (1922) the external currents, Gemmill (1920) the currents of the ephyra stage, while Henschel (1935) investigated the reactions of the oral arms alone. A study of the external and internal currents of the whole animal cleared up certain apparently conflicting details in these accounts, and disclosed several new features (Southward, 1949). It is now possible to give an account of a more complete investigation of the ciliary currents, both in the adult and in the larval stages of *Aurelia*, and to correlate some points of morphology with the currents.

In accordance with established use, the term 'ciliary currents' has been used to describe what are strictly flagellar currents. There is no ciliated epithelium as the term is understood in the higher metazoa; instead, as in most medusae (Hyman, 1940; Krasinska, 1914), a proportion of the ectodermal cells bear single flagella. In formalin-preserved material the flagella vary from 9 to 18 μ in length, somewhat longer than the cells themselves.

Observations on the living *Aurelia* were made at Liverpool in 1947 and 1948 on specimens from the Mersey estuary; at Port Erin in 1947–51 on specimens from the west coast of the Isle of Man; and at Plymouth in 1954 on specimens from the Tamar estuary. The external currents were investigated by adding suspensions of carmine or graphite particles to the water in which the specimens were kept, or by feeding with diatom cultures, mixed plankton or various proteins. The finer details of the currents were observed on specimens restricted in small dishes, but without narcosis, and were sometimes checked on isolated parts. Similar procedures often sufficed to show the internal currents, but it was sometimes necessary to inject suspensions directly into the canals and pouches.

Material was fixed in calcium-formalin, and much structure could be made out by simple sagittal sections. For more microscopic detail, standard paraffin embedding proved unsatisfactory due to shrinkage of both cells and mesogloea, and sections were cut by the gelatin-freezing method (Baker, 1944) and the glycol wax procedure (Miles & Linder, 1952). The gelatin-freezing method produced least shrinkage of the mesogloea, and was found satisfactory for grosser detail, while the glycol wax procedure was preferable for cytological detail. All material was bulk-stained with Mayer's haemalum, but sections were sometimes reinforced with iron haematoxylin and eosin.

The figures are based on camera-lucida drawings of the fixed or sectioned material, to which the details of currents were added later from rough sketches of the living material.

THE EXTERNAL CILIATION OF THE ADULT

The Umbrellar Surfaces

The cilia of both the ex-umbrellar and sub-umbrellar surfaces beat centrifugally, towards the edge of the disc. Particles and food material are trapped in mucus, which accumulates in rolls and travels gradually to the umbrella margin.

The Umbrella Margin

The margin of the umbrella, except in the region of the eight sense organs (rhopalia), bears an outer row of lappets, an intermediate row of tentacles, and an inner fold of ectoderm (Fig. 2 A); the latter, from its superficial resemblance to the velum of the hydromedusae, is called the velarium (Figs 1 and 2 A, *V*). The cilia on the tentacles beat distally, towards the tip, but the direction of travel of the material depends on the position of the tentacle. If the tentacle is relaxed and hanging free vertically the material is rejected, but if the tentacle is contracted, for example by stimulus of food, the material is caught up by the other structures of the margin. Each of the lappets has a peripherally directed current on the outer side and a centripetal current on the inner side: the result is that material collected by the lappets, and that reaching them from the ex-umbrellar surface, is passed between the lappets into the marginal groove (Fig. 2 A, *Mg*). Material collected on the sub-umbral surface is passed smoothly over the velarium and also accumulates in the marginal groove. In this groove the currents move away from the rhopalia and towards the regions of the adradial canals (Fig. 1, right half) and material accumulates beneath the adradial canals in slight widenings of the velarium, termed food pouches by Orton (1920). The marginal groove, the food pouches and the side of the velarium directed towards the marginal groove are all richly ciliated, and in section show tall columnar cells similar to those of the inner, endodermal surfaces of the oral arms.

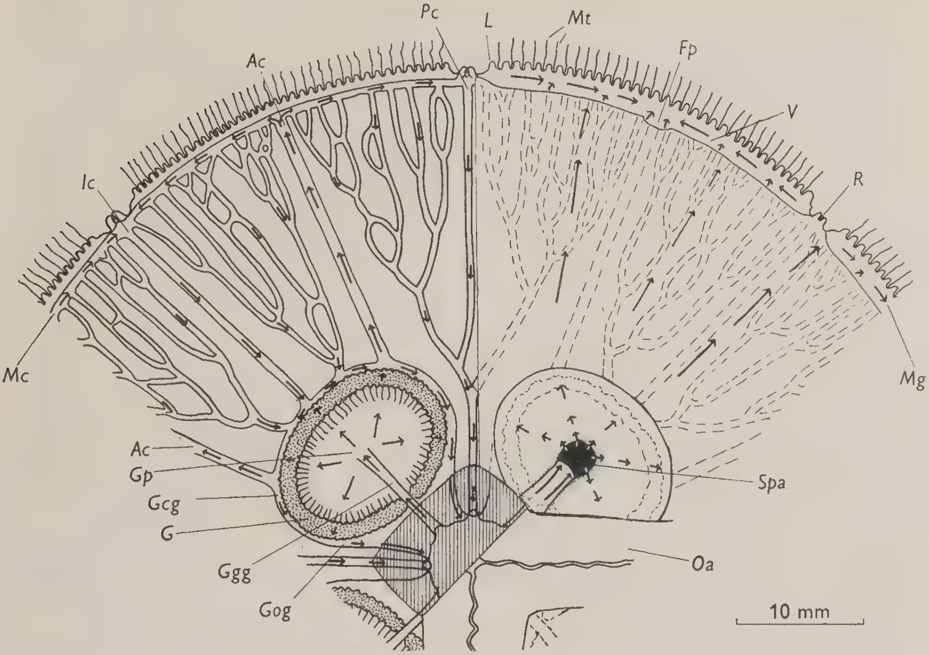

Fig. 1. *Aurelia*: part of the disc seen from the sub-umbrellar surface, with two of the oral arms removed (the cut bases hatched). The right side shows the currents on the sub-umbrellar surface and at the margin, while the left side shows the major currents in the canals and in one of the gastric pouches. *Ac*, adradial canal; *Fp*, food pouch; *G*, gonad; *Gcg*, gastro-circular groove; *Ggg*, gastro-genital groove; *Gog*, gastro-oral arm groove; *Gp*, gastric pouch; *Ic*, interradial canal; *L*, lappet; *Mc*, marginal canal; *Mg*, marginal groove; *Mt*, marginal tentacle; *Oa*, oral arm; *Pc*, perradial canal; *R*, rhopalium; *Spa*, aperture of sub-genital pit; *V*, velarium.

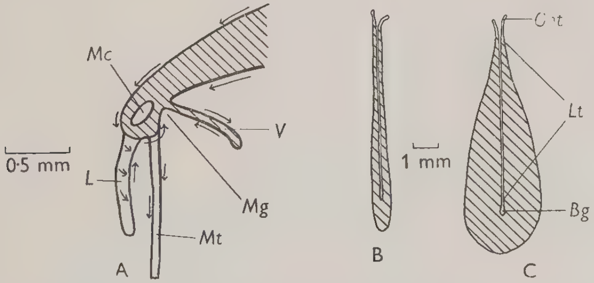

Fig. 2. *Aurelia:* diagrammatic sections of: (A) the umbrella margin; (B) the distal, and (C), the proximal regions of an oral arm. *Bg*, basal groove tract; *L*, lappet; *Lt*, lateral tract; *Mc*, marginal canal; *Mg*, marginal groove; *Mt*, marginal tentacle; *Oat* oral arm tentacle; *V*, velarium.

The Sub-genital Pits

On the outside of the pits most of the cilia beat outwards, but there is a small area on the inner side with cilia beating towards the aperture of the pit (Figs. 1, 4 B). Internally, the cilia on both the roof and the floor of the pit appear to beat centripetally towards the orifice; those on the floor set up a strong outward current, and by so doing apparently cause a compensatory current of water to enter the pit. As shown by particles in it, this current enters through the centre of the orifice with the assistance of the inward beating area of cilia (Fig. 4 B), and passes centrifugally to the periphery of the pit beneath the gonad region. The greater part of the water passes back to the aperture along the floor of the pit and issues in a thin layer around the margin of the orifice, but sometimes a smaller volume of water was seen to travel centripetally beneath the roof of the pit. This latter current may have been an artefact resulting from the inverted position of the animal during the observations or from excessive stimulation by particles. In fact, what appeared to be a cleaning reaction was displayed if many particles were present, a current of water being forced out of the pit by contraction of the sphincter-like aperture; this current momentarily reversed the normal ingoing current and effectively removed the particles.

The circulation in the sub-genital pits seems to be largely respiratory in function, especially with regard to the gonads. The latter are separated from the pits only by a single layer of thin ectoderm and the merest trace of genital epithelium.

THE INTERNAL CURRENTS OF THE ADULT

The Oral Arms

The oral arms of the adult jelly-fish are formed by elongation of the four corners of the simple mouth of the ephyra stage. They are V-shaped in section, and extend horizontally below the sub-umbrellar surface, the proximal part being supported by a thick layer of gelatinous mesogloea (Fig. 2 C), while the distal half is thinner and free to move round the margin of the umbrella (Fig. 2 B). The two halves of the V are normally held close together, apparently partly by the action of a layer of muscle fibres beneath the ectoderm, for in preserved material the arms may be gaping.

On the outer, ectodermal, surfaces of the oral arms the cilia beat towards the open margin of the V; any material collected in the mucus passes over into the groove of the V. The ciliation of the endodermal surfaces of the groove is more complex. At the extreme base there is a continuous distally-moving current, the basal groove current, which is largely excretory, for the particles carried by it have usually passed through the gastric pouches and canals (Fig. 3, *Bg*). This tract of cilia seems distinct from the remaining cilia of the groove which can act in two ways. Food material, including the mucous masses picked up from the marginal food pouches, travel proximally along the arm in a tract formed by the apposition of the two sides of the groove (Fig. 3, *Lt*), and is passed on to the gastric pouches. The width of the lateral tract involved varies with the amount of food in it; in some cases the whole of the groove, with the exception of the basal tract, may be seen moving food towards the gastric pouches. These two opposing tracts have never been seen to mix in a healthy jelly-fish, yet they are apparently separated only by the close apposition of the two halves of the V, which must nevertheless leave room for the cilia to work.

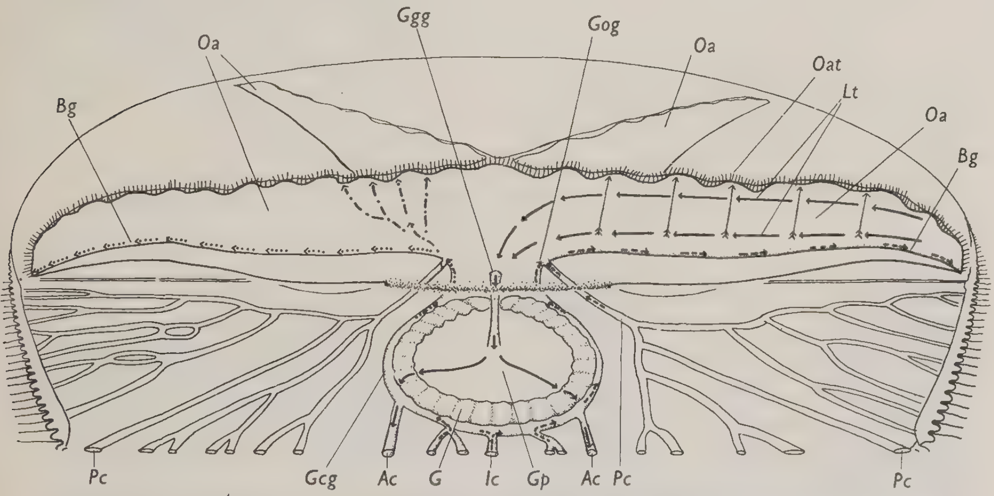

Fig. 3. *Aurelia:* oblique view of part of the disc from the sub-umbrellar surface, showing some of the currents in two of the four oral arms and in one of the four gastric pouches. The right side shows the path of food (solid arrows) in the lateral tract, and of excretory matter (broken arrows) in the basal groove, while the left side illustrates the main paths of the gametes at spawning (path of sperm in male shown by dotted arrows, path of eggs in female shown by dotted and dashed arrows). The feathered arrows represent the rejection reaction in the lateral tract. *Ac*, adradial canal; *Bg*, basal groove tract; *G*, gonad; *Gcg*, gastro-circular groove; *Ggg*, gastro-genital groove; *Gog*, gastro-oral groove; *Gp*, gastric pouch; *Ic*, interradial canal; *Lt*, lateral tract; *Oa*, oral arm; *Oat*, oral arm tentacle; *Pc*, perradial canal.

In the absence of food the lateral tract rejects nearly all inert particles placed in it. More rarely inert material may be picked up and passed a short distance proximally, but sooner or later there comes a marked change in the current, and the inert material is carried laterally to the margins of the V and rejected (Fig. 3, feathered arrows).

THE GASTRIC POUCHES

The currents in the gastric pouches are more complex than suggested by Widmark (1913), for selection mechanisms are present as in the oral arms. Food from the lateral tracts of the oral arms passes into the gastric pouches by the gastro-genital grooves (Figs. 3, 4, *Ggg*). These grooves, first noted by Goodey (1908, 1909), have two tracts of cilia beating in different directions. In the upper part of the groove, between the roof of the gastric pouch and the sides of the groove, there is an ingoing food-bearing current continuous with the lateral tracts of the oral arms (Fig. 4 B, D, *Gggu*). In the lower part of the groove, the cilia beat outwards towards the mouth, but this is not noticed superficially unless inert material is injected into the groove, when a rejection reaction occurs and material in the upper part of the groove may pass through to the lower part and be carried outwards (Fig. 4 B, D, *Gggl*). If much inert material is present in the groove a further rejection reaction may take place, the material being passed laterally to the origin of the oral arm basal tract, apparently by withdrawal of the sides of the groove from apposition with the roof (Fig. 4 A, feathered arrows).

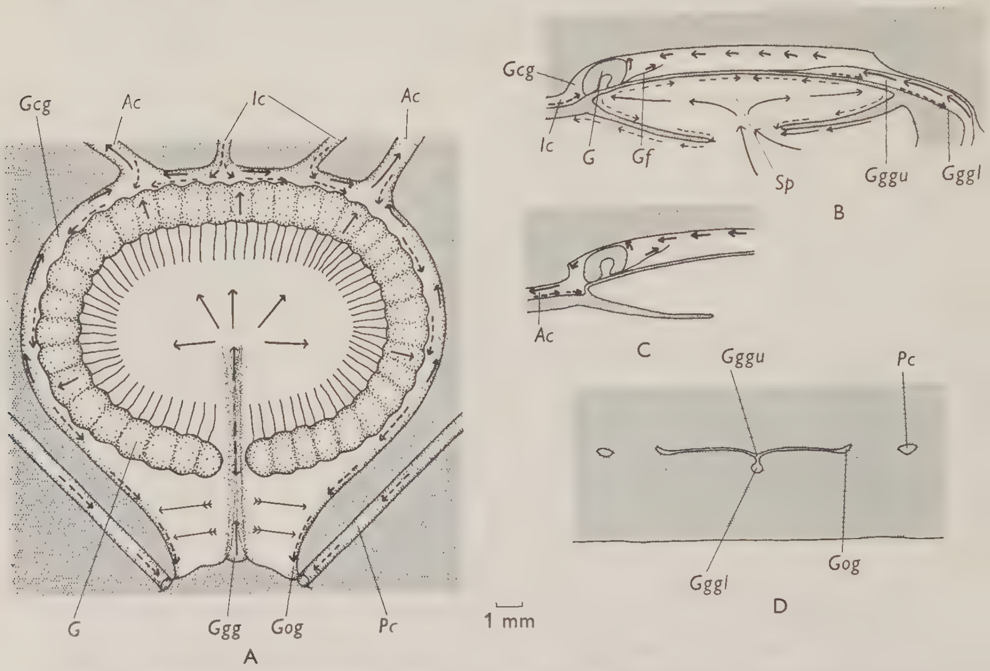

Fig. 4. *Aurelia:* A, diagrammatic view of a gastric pouch from above, with the ex-umbral surface and upper mesogloea removed; B, a radial section across the same pouch and the sub-genital pit below; C, a part section of the same pouch at the origin of an adradial canal; D, a section across the gastro-genital groove inward of the gonad. The solid arrows show ingoing currents, the broken arrows outgoing currents, the feathered arrows rejection reactions, and the thin arrows in B represent the water circulation in the sub-genital pit. *Ac*, adradial canal; *G*, gonad; *Gf*, gastric filament; *Gcg*, gastro-circular groove; *Ggg*, gastro-genital groove; *Gggl*, lower, and *Gggu*, upper, parts of the gastro-genital groove; *Gog*, gastro-oral groove; *Ic*, interradial canal; *Pc*, perradial canal; *Sp*, sub-genital pit.

Food material entering the gastric pouch by the gastro-genital groove is swept centrifugally by cilia on the roof of the pouch, and comes to rest against the gastric filaments. The cilia of the filaments beat distally, and function mechanically as a means of breaking up food masses. The separated particles pass up and over the gonads into the gastro-circular groove running round the gonads (Fig. 4 A, B, *Gcg*). Food materials travel along the roof of this groove, to the openings of the adradial canals, into which they pass. The floor of the groove is largely excretory, for indigestible matter from the interradial canals travels round it to the gastro-oral grooves (Fig. 4 A, *Gog*) along which it passes to the basal grooves of the oral arms (Fig. 3, right half). Thus the gastro-circular groove forms a further selection mechanism. Selection can also occur in the main body of the pouch, where the centripetally beating cilia on the floor can carry material to the lower part of the gastro-genital groove.

The Canals

It must be noted that the details of currents given in this paper may not apply to all specimens of *Aurelia*, since structural variation is not uncommon (Browne, 1901). This is especially true of the canals, and slight differences in the method of branching of the perradial and interradial canals can be found in practically every specimen; in some cases there may exist anastamoses between the adradials and the side branches of the other canals, which may by-pass the normal circulation.

The cilia on the roof and sides of the adradial canals beat centrifugally, and convey food from the gastric pouches to the marginal canals (Fig. 1, left half). The cilia lining the floor of the adradial canals beat in the opposite direction, and indigestible particles that have penetrated this far may be carried back to the gastro-circular groove (Fig. 4 A). Water and particles entering the marginal canal from the adradial canals pass round on either side of the adradials to the side and main branches of the perradial and interradial canals along which they travel towards the centre of the disc (Fig. 1, left half). The interradial canals open into the gastro-circular grooves, and particles are transferred to the floor tract of the latter, while the perradial canals communicate directly with the bases of the oral arm grooves (Fig. 3, right half).

So far, only one current has been detected in the interradial and perradial canals. These canals are flattened horizontally, while the adradials, which carry two currents, one above the other, have their greatest dimension vertically. In all canals the circulation seems to be assisted by the muscular pulsations of the umbrella, the walls of the canals expanding and contracting with the coronal muscles.

The Ciliary Currents of the Larval Stages

The direction of beat of the cilia remains fairly constant through the successive larval stages to the adult. For example, the locomotion of the planula is effected by cilia which beat orally (Fig. 5 A); after settlement on the aboral end, the same direction of beat now serves to accumulate food particles near the mouth (Fig. 5 B). A similar direction is maintained on the developing tentacles, which can, however, vary the ultimate direction of the particles collected by changing their posture. After strobilation the orally directed current of the scyphistoma becomes the centrifugal current of the exumbral surface of the ephyra, while the orally directed current near the mouth of the scyphistoma is represented by similarly directed currents on and near the manubrium of the ephyra (cf. Gemmill, 1920). The other external currents of the ephyra are centrifugal.

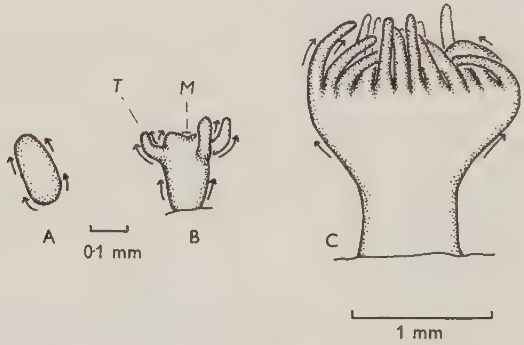

Fig. 5. *Aurelia:* A, planula, B, early scyphistoma, and C, later scyphistoma stages, showing external currents. *M*, mouth; *T*, tentacle.

The internal currents of the early ephyra are simple and adapted to the wide open gastric cavity and absence of a marginal canal (Fig. 6 A). The cilia on the roof of the gastric cavity beat centrifugally, and those on the floor centripetally, but much swirling is caused by the gastric filaments and by the muscular contractions of the animal. The more complicated canal system and circulation of the later stages develops with the expansion of the area of the

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Legend to Fig. 6.

Fig. 6. *Aurelia:* A, an early ephyra, the currents of which were reported by Gemmill (1920); B, a slightly older ephyra than A, showing the origin of the marginal canal; C, 10 mm. stage, with marginal tentacle and velarium developing; D, a slightly larger view of an adradius of the specimen in C, but from the ex-umbral aspect; E, 15–20 mm stage, with oral arms and gastric pouch. All except D from the sub-umbrellar surface. *Ac*, adradial canal; *Bg*, basal tract; *Gf*, gastric filament; *Gcg*, gastro-circular groove; *Ggg*, gastro-genital groove; *Gog*, gastro-oral groove; *Gp*, gastric pouch; *Gprg*, gastric pouch ring groove; *Ic*, interradial canal; *L*, lappet; *Lt*, lateral tract; *Mc*, marginal canal; *Mn*, manubrium; *Mt*, marginal tentacle; *Pc*, perradial canal; *Pt*, primary tentacle; *R*, rhopalium; *V*, velarium.

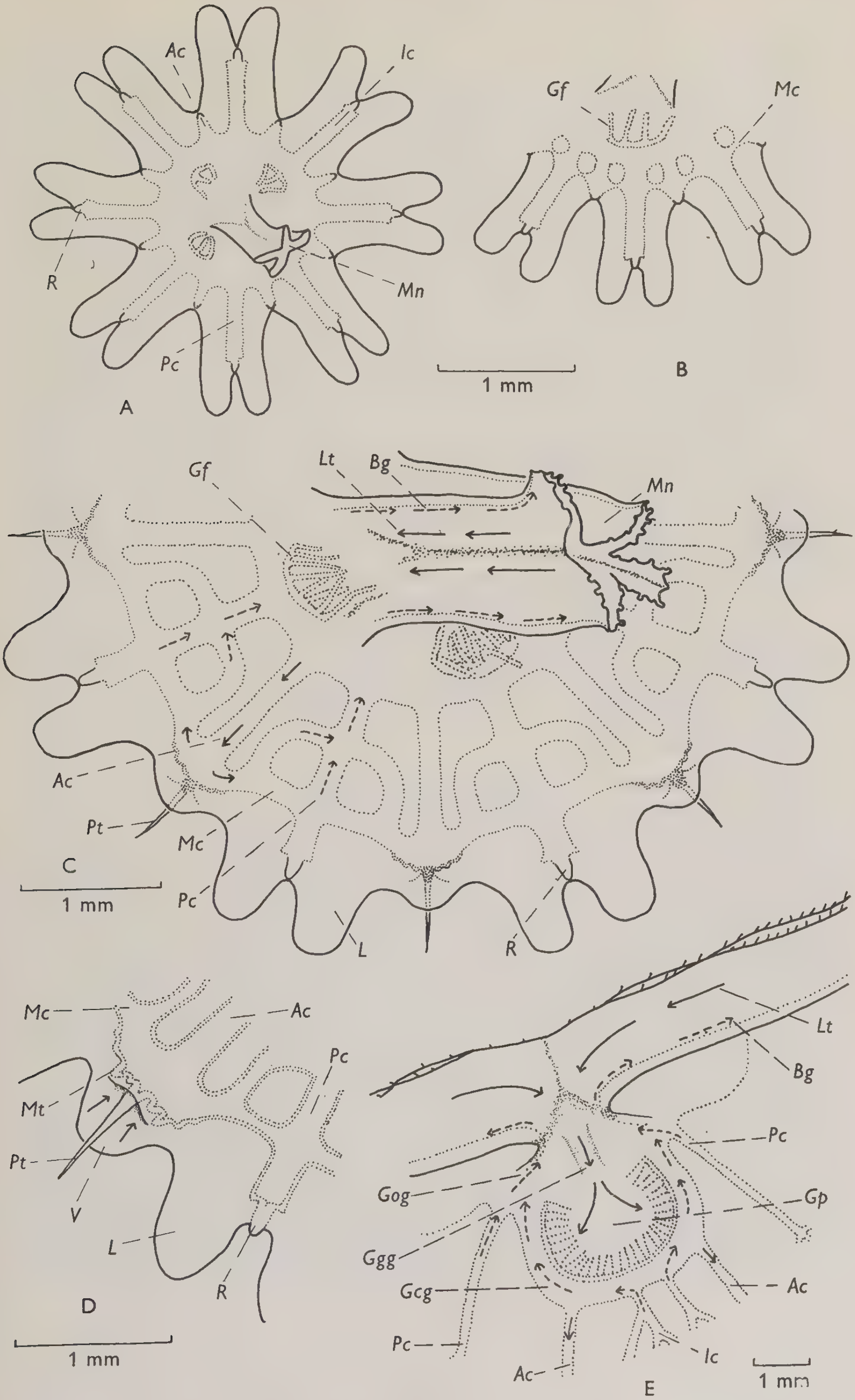
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Fig. 6.

disc (Fig. 6 c). The marginal canal arises by connexion between the outer parts of the radial canals (Fig. 6 B, C): it is significant that the main outward currents from the gastric cavity should pass along the adradial canals, as a large part of the disc arises by growth at the adradial margins, between the rhopalia. At a diameter of about 10 mm most of the ciliary currents resemble those of the adult (Fig. 6 c), except that food masses collected by the umbrellar surfaces accumulate at the margin on both the rhopalial lappets and on the developing velarium. The latter arises as a series of small lappets at the adradial margins above the groups of developing tentacles (Fig. 6 D).

Food masses are licked off the lappets of the ephyra by the mobile manubrium, the internal currents of which agree with those of the oral arms of the adult: an inward current over most of the inner surfaces, and an outward current in the grooves at the four corners. The oral arms are fully developed at a diameter of 15–20 mm, and show the currents and reactions of the adult (Fig. 6 E). The gastric pouches, however, are not complete at this stage, although the gastro-circular groove functions as in the adult. Incipient gastro-genital grooves can be made out (Fig. 6 E, *Ggg*), and food usually passes down them to the gastric pouches, but occasionally material may be seen to enter the pouches on either side of the grooves.

At 20 mm diameter all the external currents resemble those of the adult, except that sub-genital pits are not yet present. The stage at which these pits arise has not been noticed in the present work, and does not appear to be known (Hyman, 1940). It seems likely that the pits develop together with the gonads, with which they are intimately concerned.

THE MODE OF FEEDING

Experiments have amply confirmed Orton's (1922) evidence of the importance of the ciliary mechanisms in food collection and reinforced the view that the adult jelly-fish is largely microphagous. Although the larval stages feed both by ciliary mechanisms and by muscular macrophagy as found by Gemmill (1920), it should be pointed out that the food they consume is similar in size to that eaten by the adult. In other words, the difference in feeding habits between the ephyra and the adult reported previously (Southward, 1949) is due merely to the growth of the jelly-fish: the food organisms are only relatively smaller in the adult stage.

The path taken by food in the adult has been described already in the separate sections on external and internal currents, but may be briefly recapitulated. Organisms are collected in mucus on the umbrellar surfaces and passed to the marginal groove where they accumulate in the food-pouches. The masses of food and mucus are licked off the margins of the umbrella by the tips of the oral arms, the fringing tentacles of which contract inwards and hold the food masses until they are taken up by the lateral tracts. The food is passed along the lateral tracts to the gastro-genital grooves, by way of which it enters the gastric pouches. After being separated by the gastric filaments the food particles are distributed along the canals. Digestion has not yet been studied in *Aurelia*, but it seems possible that, as in *Cassiopeia* (Smith, 1936), the gastric filaments can produce some extracellular enzymes.

The collection of food can be observed directly by placing *Aurelia* in a suspension of plankton organisms: indirectly, the type of food being collected can be determined by examination of the mucous masses in the food pouches. The ex-umbrellar surface plays least part in the process of food collection, since large copepods and other organisms are swept off during pulsation of the bell. The main collecting takes place on the sub-umbrellar surfaces, on the oral arms and at the margin of the umbrella, the action of swimming causing a current of water to pass over these parts.

A specimen 10 cm in diameter cleared all the coarse plankton from 700 ml. of water in less than an hour, but much larger volumes of sea water should be passed over the external surfaces in nature. *Aurelia* easily picks up small copepods (e.g. *Tigriopus*) on the external surfaces, and at 15° C these can reach the food pouches within 10 min, the oral arms in 12 min and the gastric filaments in 50 min. Material picked up by the oral arms directly reaches the gastric pouches more quickly, sometimes within 10 min.

Examination of the mucous masses in the food pouches of freshly captured specimens usually shows plankton typical of the place of capture. Thus, the food pouches of *Aurelia* from Port Erin Bay in early June showed principally copepods, which were then present in great abundance in the sea. Specimens from the River Mersey had large amounts of detritus (probably silt and sewage), in addition to diatoms, ciliates, flagellates and some crustacean eggs, in the food pouches. The pouches of further specimens from a tide pool near the mouth of the Mersey estuary contained, in addition to large amounts of detritus, many *Noctiluca* and some balanoid cyprids, both common in the local plankton at the time. Other specimens kept in tanks at Port Erin had collected plankton typical of the local sea-water supply, namely diatoms, algal fragments, polychaete larvae and harpacticid copepods. *Aurelia* placed in pure cultures of the diatom *Nitzschia* or the copepod *Tigriopus* rapidly accumulated these organisms in the food pouches, and later, in the gastric pouches.

The evidence for plankton feeding, and microphagy in general, is thus very strong. Attempts to show macrophagy in the adult have always failed, as food particles above 5 mm diameter are always dropped or rejected from the margin or the oral arm grooves. A further limitation of the size of food masses is imposed by the narrowness of the gastro-genital grooves. Although, theoretically, the mouth communicates with a gastric pouch by the whole width of the tract between the two gastro-oral grooves (Fig. 4 A), in practice food enters a pouch only along the gastro-genital grooves.

Selection mechanisms play an important part in the feeding processes of microphagous animals, and *Aurelia* shows such mechanisms at several stages in the food path. The tentacles may accept or reject according to their position relative to the marginal groove, and their degree of contraction, but the oral arms constitute the major selective device outside the gastric pouches. As Henschel (1935) showed, the arms have a well-marked chemical sense, and respond to proteins and nitrogenous substances, but not to carbohydrates or inorganic particles. The reaction to food masses, plankton, or mussel extract is seen first as a contraction of the fringe of capitate tentacles (which bear batteries of nematocysts), and the material is then taken up by the lateral tract. Materials such as carmine, graphite or starch grains cause little or no contraction of these tentacles and are not usually taken up by the lateral tract. If such indigestible particles are taken up by the lateral tract (possibly as a result of the presence of unseen food organisms in the water) they are later rejected before reaching the gastric pouches, by a lateral movement in the tract (Fig. 3, right half). This rejection reaction can often be halted, and the material caused to be taken up again, by stimulation with proteins (e.g. egg albumen), and if the stimulus is sufficiently strong the indigestible material may pass to the gastric pouches. On the other hand, further addition of much indigestible material to the lateral tract may cause the arm to reject the whole contents of the tract, including any food masses that may be present.

In the absence of obvious structural features, such as the grooves in the throat of the anemone *Metridium* (Elmhirst, 1925; but cf. Parker & Marks, 1928), it might be thought that the rejection reaction shown by the oral arms of *Aurelia* was due to a change in direction of beat of the cilia. Unfortunately it has not been possible to see the direction of beat of a cilium directly *in situ* in the arm, and it is difficult to expose a single inner surface without damaging the cilia. However, it is possible that the 'reversal' is only apparent. Thus, the normal ingoing current of the lateral tract creates its own 'canals' by apposition of the sides of the groove around the food or other particles; relaxation of the muscle fibres partly responsible for this apposition would allow these canals to communicate with the exterior, and the rejection might merely result from the material taking the easier path to the exterior. Further investigation of the rejection reaction is necessary before this theory can be accepted.

The other selection mechanisms occur in the gastric pouches and in the adradial canals. That described for the gastro-genital groove (p. 206) is probably of chemical nature like the oral arm reaction, but the remainder may be mechanical, serving to sort out the heavier, and therefore presumably inorganic particles from the food.

Although scattered batteries of nematocysts are present on the ex-umbrellar surface of *Aurelia*, they appear to play little part in food capture in the adult; their penetrating powers, assessed on human epidermis, are much poorer than those of other semaeostome medusae. In the ephyra, however, the nematocysts help to hold captured organisms on the lappets (Gemmill, 1921) until they can be licked off by the manubrium. The smallest ephyra stage (Fig. 6 A) has been seen to hold and later ingest in this way as many as six nauplii of *Balanus balanoides*, almost simultaneously: at the same time, masses of smaller organisms collected by the ciliary currents were also eaten. Ephyrae of up to 10 mm diameter were found feeding on the newly hatched plaice larvae in the fish-hatchery at Port Erin, and one specimen accepted up to three larvae, one after the other, in the same way that the earlier stage dealt with nauplii. In these stages the manubrium showed marked selection mechanisms, and, like the oral arms of the adult, rejected indigestible particles.

The Currents and Reproduction

As is well known (Agassiz, 1860; Hargitt & Hargitt, 1910), the gametes of *Aurelia* issue from the gonads and gastric pouches along the oral arms; the eggs, which are already fertilized, become enclosed in pockets of the inner, endodermal, surfaces of the oral arms, near the margins of the grooves, where they develop to the planula stage. Goodey (1908, 1909) believed that the eggs and sperm left the gastric pouches by way of the gastro-genital grooves ('gonadial grooves'), and was able to show their presence in this channel in preserved and sectioned material. However, in the course of the present work it was frequently observed that mature female *Aurelia*, after handling, passed eggs and planulae round the canals, and their presence in any part of the system is not remarkable. Widmark (1913) observed the passage of gametes along the gastro-oral arm grooves ('eck-canalen'). During the present study only four specimens have been seen to spawn, two males and two females, but in all the gametes likewise issued along the gastro-oral arm grooves (Fig. 3, left half). In the males the bulk of the sperm was passed to the basal grooves of the oral arms, in which it travelled to the tips of the arms, where it was ejected in mucous masses. The females differed in that the eggs travelled only a short distance in the basal grooves, and were quickly passed to the lateral tracts, in which they moved laterally, quite like rejected material, to the margins of the grooves.

Internal fertilization is presumably effected by the females picking up the sperm masses ejected by the males, and passing them to the gastric pouches in the normal feeding currents. Sperm masses were, in fact, found in the marginal food pouches of a female kept in the same tank as a spawning male. The process is probably assisted by the shoaling that frequently occurs during the breeding period (Agassiz, 1860; Southward, 1954).

Comparison with Other Jelly-Fish

In its restriction to a largely microphagous habit, and in its reliance upon ciliary mechanisms for food transport and internal circulation, *Aurelia* resembles the rhizostome medusa *Cassiopeia* (Smith, 1936). However, studies of other semaeostome and rhizostome mudusae, not completed, show that the two groups have many common features of physiology. Thus, the centrifugal currents of the ex-umbrellar and sub-umbrellar surfaces of *Aurelia* are present, slightly modified, in *Cassiopeia* and apparently in the semaeostomes *Chrysaora* and *Cyanea*, but in these species the currents play little part, if any, in food collection. In all these species, and in *Rhizostoma* as well, the inner surfaces of the oral arms show similar currents and functions: an outgoing current in the base of the groove (in rhizostomes that part of the oral arm canal nearest the sub-umbrellar surface) and an ingoing current, often capable of rejection, on the remaining surfaces.

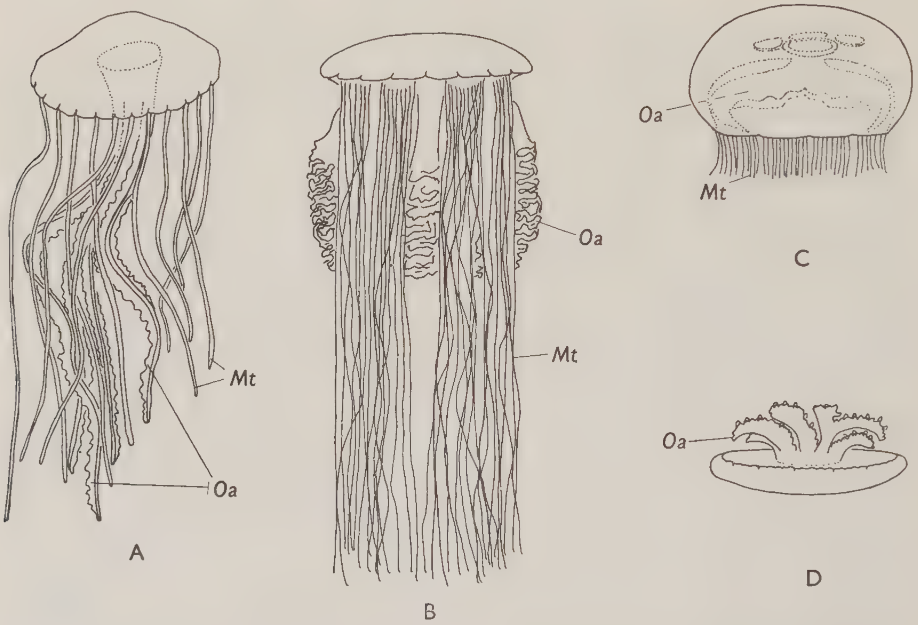

Fig. 7. Diagrammatic sketches of A, *Chrysaora*, B, *Cyanea*, C, *Aurelia* (in swimming position), and D, *Cassiopeia* (in attached posture), all in side view to show the relative proportions of the marginal tentacles (*Mt*) and the oral arms (*Oa*). A, C and D to the same scale, about $\frac{1}{3}$ natural size; B, approximately one-half the scale of the rest.

Internally the species so far studied have a centrifugal current on the roof of the gastric pouches or gastric cavities, and a centripetal current on the floor. *Cyanea* and *Chrysaora*, however, differ from the rest in their lack of a marginal canal or plexus, and hence show no regular circulation in the radial canals (Widmark, 1911): in this they resemble the ephyra of *Aurelia*, in which the circulation is also partly ciliary and partly muscular. These two species differ from the adult *Aurelia* and the rhizostomes in possessing a wide central gastric cavity communicating freely with the oral arms, and might be said to be less specialized. However, they diverge widely from the possibly ancestral ephyral type in another direction, for the oral arms and marginal tentacles are greatly elongated—specializations apparently directed towards a drift-net habit of feeding (Fig. 7).

If *Chrysaora* (Fig. 7 A) is the least specialized of these species (cf. Agassiz, 1860), *Aurelia* can be regarded as an intermediate stage in the direction of the rhizostomes, by reduction of the marginal tentacles and by the greater reliance placed on ciliary currents for food collection: the rhizostomes themselves have no tentacles, and the oral arms alone gather food. The final development of this trend may perhaps be found in *Cassiopeia*, which is sessile and employs the pulsations of the bell to pass water over the oral arms (Fig. 7 D). On the other hand, *Cyanea* shows greater adaptation than *Chrysaora* for the drift-net type of feeding; the enlarged frills of the oral arms (Fig. 7 B) must be necessary to cope with the increased number and catching area of the tentacles, to ensure that organisms captured are transferred to the arms.

This paper owes much to the late Prof. J. H. Orton, F.R.S., who suggested the investigation to me, and who gave freely of advice and encouragement. Mr F. S. Russell, F.R.S., kindly advised me on several matters, and Dr D. J. Crisp and Dr D. Atkins helpfully criticized the typescript. I am indebted to Captain Nicholson, the Lancashire and Western Sea Fisheries Committee bailiff for Liverpool, Mr A. Cregeen, the Port Erin Marine Biological Station boatman, and Mr A. Briggs, the Marine Biological Association collector, for the supply of living material. Mr T. R. Tozer kindly supplied well-preserved specimens of all stages.

I am grateful for financial assistance from the University of Liverpool, and the Department of Scientific and Industrial Research.

SUMMARY

The jelly-fish *Aurelia aurita* possesses external and internal ciliary currents that play a large part in food collection and in the transport of food, reproductive products and excretory matter.

Adults feed on relatively small organisms, which are collected in mucus on all external surfaces and eventually passed to the inner surfaces of the oral arms.

The inner surfaces of the oral arms bear two ciliated tracts which operate simultaneously in opposite directions. The lateral tract carries food materials proximally towards the gastric pouches, but is capable of rejecting inedible matter. The basal tract carries excretory matter distally, away from the gastric pouches and canals to the exterior.

Rejection reactions are also found in the gastric pouches and radial canals, parts of which have currents moving in opposite directions on the roof and on the floor. These opposing currents appear to be derived from the system in the ephyra stage, where the circulation in the wide gastric cavity and blind-ending

canals is maintained partly by centripetal currents on the floor and centrifugal currents on the roof.

The directions of the main currents remain constant throughout the larval stages to the adult, although slight variations are introduced by morphological changes. The currents also remain the same during spawning, when the eggs and sperm leave the gastric pouches by the normal excretory path.

Many of the ciliary currents found in *Aurelia* are present in other semaeostome and rhizostome medusae, but only in *Aurelia* do the umbrella surfaces and currents play a large part in food collection. Some of the major morphological differences in the Scyphomedusae can be related to the different feeding habits of the various species.

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BLOOD PERFUSION OF THE KIDNEY OF *LOPHIUS PISCATORIUS* L.

III. ACTION OF CO_2, CYANIDE AND FLUORIDE

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(Text-figs. 1 and 2)

Proceeding with our studies on urine secretion of the aglomerular kidney of *Lophius piscatorius* L., in the Plymouth Laboratory (Brull & Nizet, 1953; Brull, Nizet & Verney, 1953; Brull & Cuypers, 1954*a*, 1954*b*) and using the method of blood perfusion we described previously, we attempted to study the influence of CO_2, and of cyanide and fluoride on water secretion and on Mg concentration, which seems to be the most characteristic function of that kidney.

METHODS

Two experiments were carried out with CO_2, three other experiments with cyanide, and two attempts were made with fluoride, the first one on a preparation used for cyanide, after recovery from that intoxication (Expt. G).

A sufficiently large kidney was perfused with heparinized blood from a pool obtained from several *Lophius*, a pool which was large enough to wash the preparation after removal of the modified or toxic blood, and to replace it by a fresh supply.

The drops of urine were registered, and after a constant or an increased urine flow was noticed, either blood in equilibrium with a different gas mixture replaced the previous one, or the toxic substance was added, dissolved in a small amount of Ringer and neutralized.

Whenever blood was removed, the circulation was stopped for a very short time, which, with practice, could be reduced to 15 sec. As soon as the effect of the toxic substance or of the modified blood was clearly established, the preparation was washed out with fresh blood, and then new blood from the same pool was put in.

Rough determinations of magnesium were made on one drop of plasma or diluted urine, with the 'Tüpfelmethode' of Feigl (1931), using titane yellow as reactive. The margin of error in these determinations is ± 20 %.

EFFECT OF CO_2

In a previous publication (Brull & Cuypers, 1954*a*), we have shown that the blood of *Lophius piscatorius* contains between 1·87 and 2·35 g of haemoglobin. Because of this low content, and of the fact that ferricyanide coagulates this kind of blood, determinations of the O_2 content are inaccurate in arterial blood, and practically impossible in venous blood, from which no measurable volume of oxygen is liberated in the Van Slyke apparatus.

Yet there remains no doubt that the kidneys of *Lophius*, receiving venous blood only, live on a very low oxygen tension. If the blood is oxygenated when perfusing these kidneys, there is no change in the order of magnitude of urinary volume or concentration (Brull, *et al.* 1953). We concluded therefore that the oxygen requirement of the *Lophius* kidney is very light.

Owing to the fact that in our previous perfusion experiments the artificial blood circulation was in contact with air or with oxygen, there resulted a progressive loss of CO_2 and a rise of pH. These alterations were not accompanied, even in the long run, by a parallel change in the water output or in the concentration capacity for Mg, except for a slow decrease of urine volume when the preparation becomes too old. Thus, the secretion is not affected by enriching the blood with O_2 and impoverishing it in CO_2.

It seemed interesting to find out whether *an increase* in CO_2 would have an influence, especially as it is well known that the Bohr effect is of high magnitude in the blood of marine teleosteans (Florkin, 1944, p. 86). This was soon confirmed by the aspect of the blood when equilibrated with a mixture of 5% CO_2 and 95% O_2; it was much darker than any venous blood we had previously seen from *Lophius*.

Fig. 1 (Expt. H and I) illustrates the results of two experiments during which kidneys were successively perfused (1) with blood in equilibrium with air; (2) with blood in equilibrium with 5% CO_2 and 95% O_2; (3) with the same as in the first.

The perfusion pressure was constantly kept at 200 mm water in the first perfusion, and at 300 mm in the second, as we know from our previous work (Brull & Cuypers, 1954*b*) that these figures are above the optimum required to produce maximum urine output.

It is obvious that high CO_2 tension produces two effects: (1) an important reduction in the flow of urine, which is totally reversible, and (2) a vasoconstriction which is not reversible after 1 hr.

Since the urine flow recovers after removal of the CO_2-enriched blood, while the blood flow remains low, one may conclude that the two phenomena are independent of each other. This is not surprising, when considering that the blood flows, even reduced by the CO_2, are still above the optimum flows, according to our previous experiments.

Now, which is the factor responsible for the reduction in the urine output: the drop of available oxygen, the CO_2 tension itself, or the drop of pH? The question remains open.

The following experiments may shed some light on the problem.

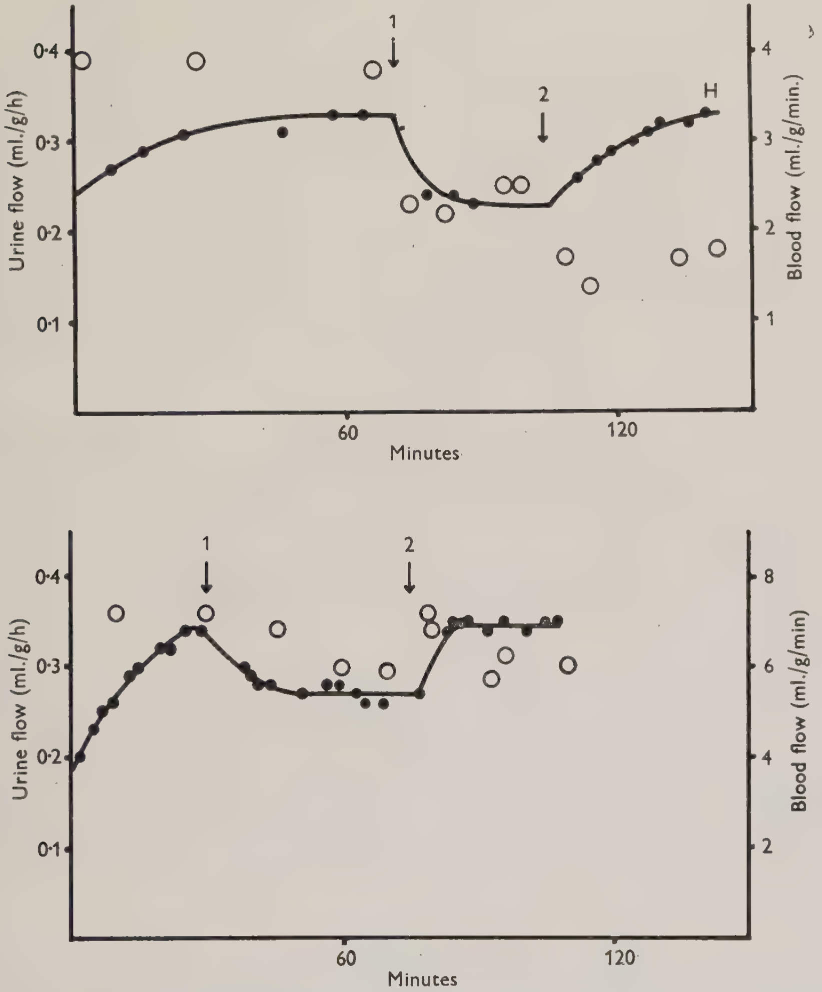

Fig. 1. Upper curve. Expt. H: a kidney of 7 g is perfused with blood oxygenated with air. At $\downarrow^{1}$ blood in equilibrium with carbogen replaces the air-oxygenated blood. At $\downarrow^{2}$ back to air-oxygenated blood. Perfusion pressure constant at 200 mm water. Lower curve. Expt. I: a kidney of 11 g is perfused with blood oxygenated with air. At $\downarrow^{1}$ blood replaced by blood in equilibrium with 5 % CO_2–95 % O_2. At $\downarrow^{2}$ back to previous blood. Perfusion pressure constant at 300 mm water.

Effect of Cyanide

In the first experiment (Fig. 2, Expt. E), the amount of cyanide given is apparently too large, there being practically no recovery after removal of the poison.

In Expt. F (Fig. 2) recovery is better but not complete. In Expt G only 8 mg KCN were added to 100 ml. of circulating blood (1·3 M/1000). This concentration reduced the urine output to one-fifth of its former level, and there was a sharp drop in the Mg concentration in the urine, from 150 mg %, down to 30 mg. Although the washing out of the kidney may not have been complete, these figures are sufficient to show that independently of the water secretion, the mechanism of which is put out of action by cyanide, its power of Mg concentration is also affected. Not only is there a drop of Mg excretion due to the decrease or arrest of urine flow, but also a marked decrease in the concentration power of the kidney for Mg. Both phenomena are reversible when the amount of cyanide is 1·3 M/1000 KCN.

Effect of Fluoride

In Expt. G (Fig. 2), after the urine volume and Mg concentration had come back to approximately their original level, 350 mg NaF were added (at arrow 3), the volume of the blood being about 150 ml. There is an immediate and sharp drop in urine output, right down to one drop in 10 min, after 16 min; the last drop of urine taken from the proximal end of the ureteral cannula shows that the Mg concentration has dropped from about 160 mg % down to 40 mg.

Expt. K (Fig. 2) is a second attempt with fluoride. When the urine flow is steady, 0·5% NaF are added (65 mg in 130 ml. of blood). Urine volume steadily decreases, down to less than 50% of the original level, after 40 min. Mg in urine drops from 160 mg down to 60 mg %. The fluorized blood is then removed, and washed out with fresh blood; this experiment, however, was stopped by a subcapsular haemorrhage in the kidney.

Discussion

It is known, through Warburg's experiments (1914), that cyanide stops or reduces some oxidative processes in the cells by blocking the iron. We shall not go into the extensive literature on the mechanism of action of HCN and of fluoride. Both chemicals reduce aerobic respiration, and fluorides especially affect the carbohydrate cycle, namely by an inhibition at the level of pyruvic acid (Peters, Rydin & Thompson, 1935; Melrose & Terner, 1953). Their effects on water secretion and Mg concentration add new evidence in favour of the concept that these kidney activities are really active processes involving energy derived from the carbohydrate cycle and oxygen consumption. Although

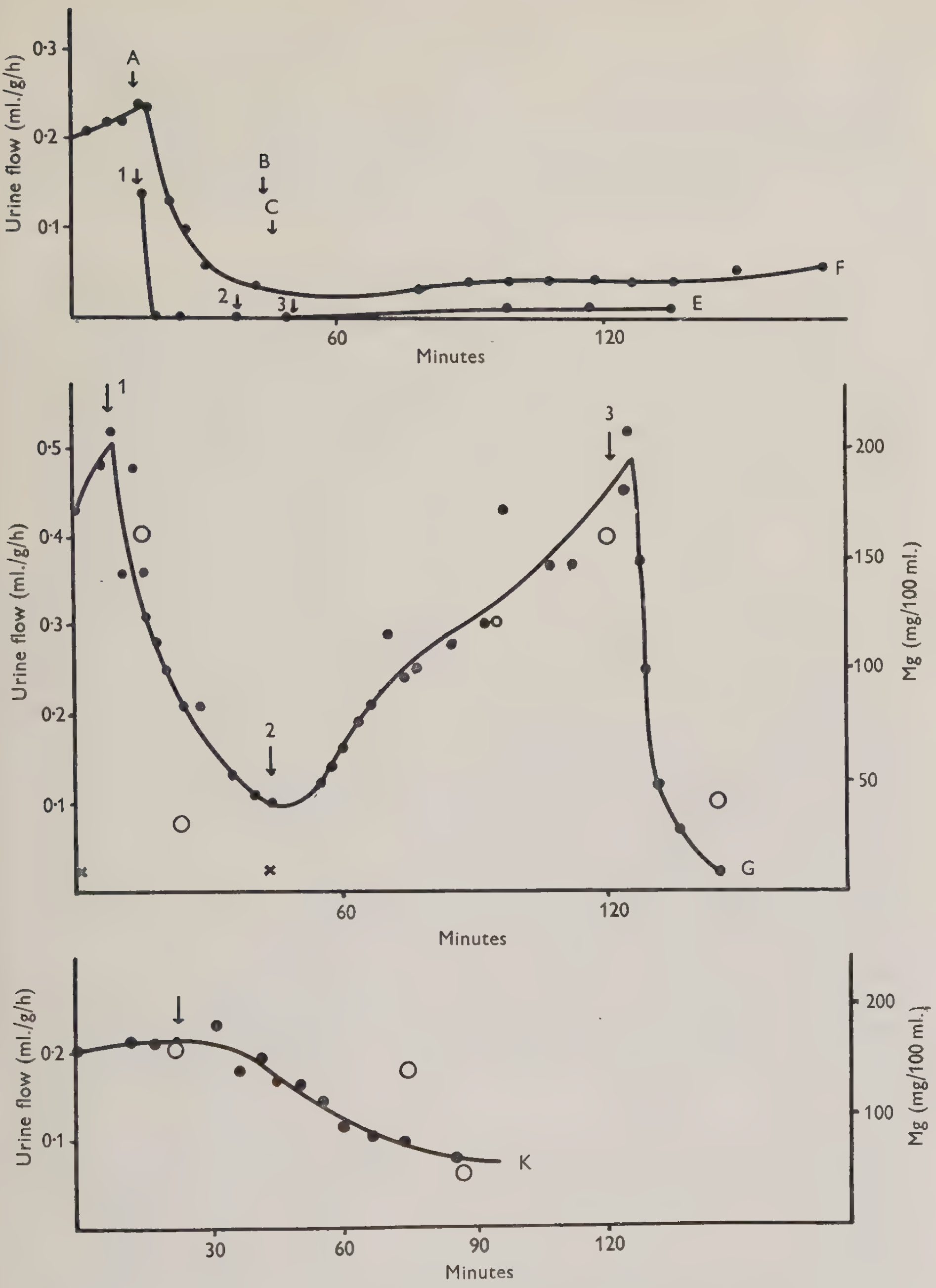

Fig. 2. Expts. E, F, G, K. Expt. E (lower curve): 160 mg KCN are added to 175 ml. of blood at 15 min (arrow 1). At 27 min the cyanided blood is removed; the preparation is washed with 100 ml. fresh blood (arrow 2). At 49 min this blood is removed and 150 ml. new blood put in (arrow 3). Expt. F (upper curve): at 14 min (arrow *A*) 20 mg KCN added to 130 ml. circulating blood. At 43 min (arrow *B*) the cyanided blood is removed. At 45 min circulation removed with 210 ml. fresh blood from which the fifty first ml. from the renal outflow are discarded. Expt. G: at the first arrow 8 mg KCN are added to 100 ml. circulating blood. At arrow 2, cyanided blood removed; the preparation is washed with 50 ml. fresh blood and new blood from the same pool is put in. At arrow 3, 350 mg NaF are put in. Expt. K: at the arrow, 65 mg NaF are added to 130 ml. circulating blood.

there is much evidence that these kidneys require relatively little oxygen, they do require aerobic processes. It is possible that by greatly lowering the oxyphoretic power of the blood, due to an important Bohr effect, CO_2 acts on the kidney by bringing the oxygen tension down below the minimum requirement.

SUMMARY

Kidneys of *Lophius piscatorius* L., perfused with heparinized blood, respond to a high tension of CO_2 in the blood by a drop in urine flow and in their capacity for concentrating Mg. At the same time, but independently, CO_2 reduces the blood flow. Both cyanide and fluoride may stop or reduce water secretion and Mg concentration, according to the concentration used, showing that both these activities are dependent on aerobic processes and presumably on the integrity of the carbohydrate cycle.

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PHOSPHORUS AND SILICON IN SEA WATER OFF PLYMOUTH DURING 1954

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(Text-figs. 1–4)

Analyses of water collected during 1954 at the International Hydrographic Station E1 (lat. 50° 02′ N., long. 4° 22′ W.) are here reported in the same form as in a previous report (Armstrong, 1954). The methods of collection and analysis of samples were unchanged.

There are no observations below 30 m. at the end of the year because of a mishap with the sampling gear in November, and because bad weather curtailed work at sea in December.

TEMPERATURE AND SALINITY

The vertical distribution of temperature during the year is shown in Fig. 1. The water column showed vertical uniformity until May. The minimum surface temperature recorded was 9·0 on 12 March. The mean salinity increased between 19 January and 16 February from 35·31 to 35·37. The significance of this small change is discussed below. On 10 May, after rough weather there was only 0·5° C. difference between the top and bottom of the water column, but on 19 May the difference was 2·3° C and there was a marked thermocline at 30 m. This thermocline remained at 25 to 30 m during the summer, but had broken down by 11 October. Summer temperatures in the upper layers were unusually low, the surface maximum of 14·92° C being recorded on 14 September. The observations for 11 August and 14 September are given in full in Table I, from which it may be seen that marked temperature increases took place. Those in the deeper water are larger than in the upper layers and cannot be put down to vertical mixing. The sharpness of the thermocline remains unimpaired. A small but significant increase in salinity occurred. The weather records from Mount Batten Station show that mean air temperatures (at Plymouth) in the interval lay between the sea surface temperatures at E1 observed on 11 August and 14 September, and that hours of sunshine were a little less than average. Although direct absorption of solar radiation causes some warming of the sea surface irrespective of the temperature of the air, it is improbable that the observed rises in temperature throughout the water column could have occurred locally. The change therefore must have been caused by a displacement of water masses on a considerable scale.

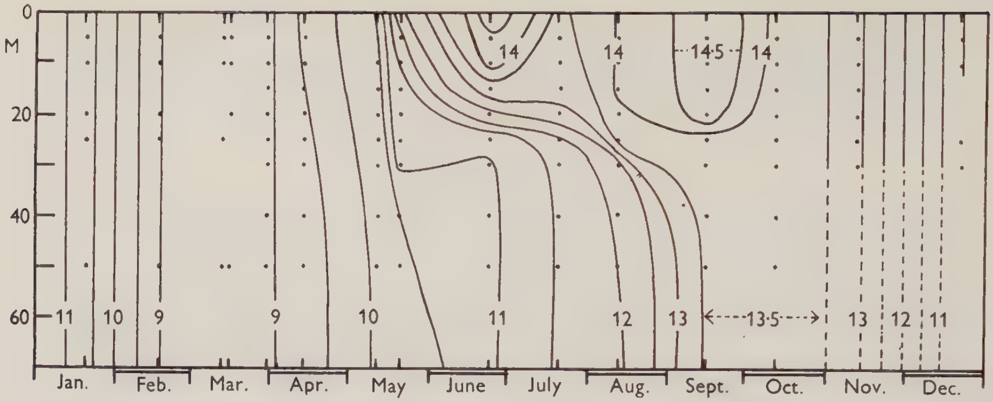

Fig. 1. Vertical temperature distribution at International Hydrographic Station E 1, 1954. Contour lines at 0·5° C intervals.

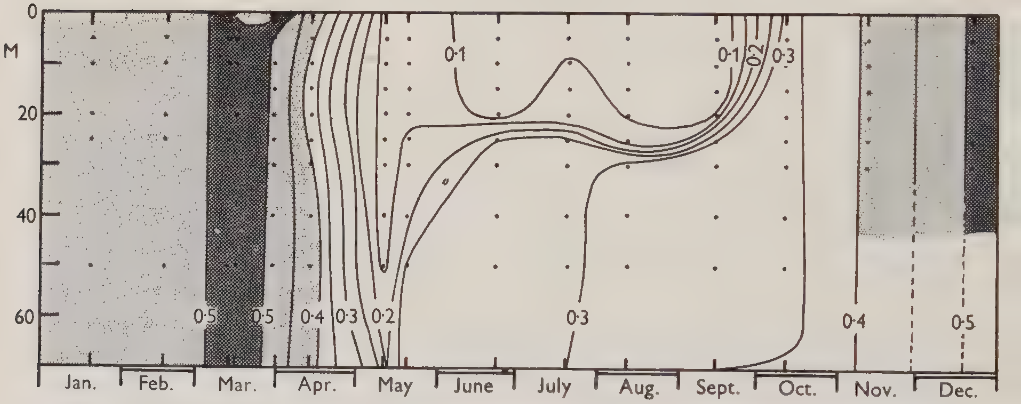

Fig. 2. Vertical distribution of phosphate as μg atom P/l. at International Hydrographic Station E 1, 1954. Contour lines at 0·05 μg atom P/l. intervals.

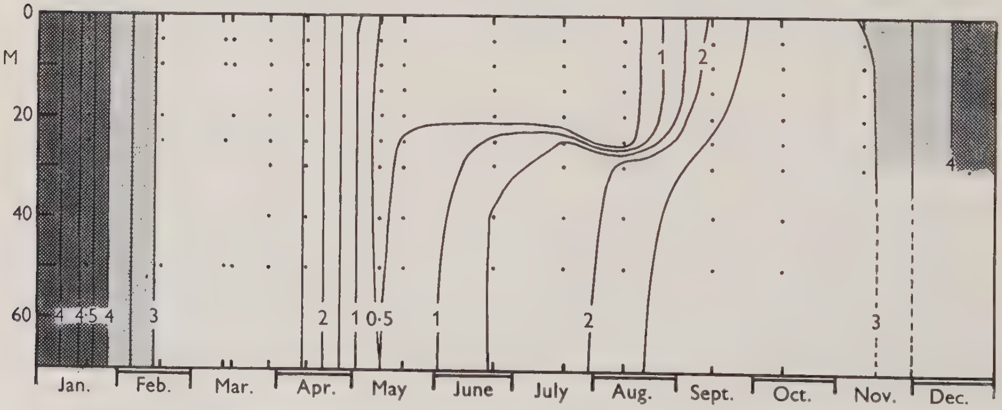

Fig. 3. Vertical distribution of silicate as μg atom Si/l. at International Hydrographic Station E 1, 1954. Contour lines at 0·5 μg atom Si/l. intervals.

During the whole of this period of 34 days a weak south-westerly air stream flowed over the area, becoming a strong south-westerly breeze (18–26 knots) for 2 days on 9–10 September. The weather remained cool and remarkably equable. The mean temperatures with standard deviations were

Max. day temp.	17·3 ± 1·3° C
Min. night temp.	12·0 ± 2·0° C
Overall mean	14·7° C

Mean hours of sunshine were 5·46 h/day. The average for this period is estimated as about 5·9.

After the breakdown of the thermocline, temperatures decreased as is normal. On 21 December the upper 10 m. was colder and less saline than at 25 and 30 m (e.g. 5 m 10·48° C, 34·76‰ S; 25 m 11·07° C, 35·10‰ S) which suggests the presence of coastal water after the heavy rainfall of late November and early December.

TABLE I. TEMPERATURE AND SALINITY AT INTERNATIONAL HYDROGRAPHIC STATION E1, 11 AUGUST AND 14 SEPTEMBER 1954

Depth (m)	Temperature (° C)		Salinity (‰)	
	11 Aug.	14 Sept.	11 Aug.	14 Sept.
0·5	14·05	14·92	35·24	35·25
5	14·05	14·87	35·21	35·25
10	14·05	14·87	35·24	35·27
15	14·03	14·87	35·24	35·27
20	13·99	14·77	35·25	35·27
25	13·98	13·70	35·25	35·30
30	12·38	13·59	35·29	35·35
40	12·22	13·57	35·30	35·35
50	12·00	13·54	35·29	35·35
70	11·91	13·53	35·33	35·33

PHOSPHATE

The vertical distribution of phosphate is shown in Fig. 2, and values at 10 and 50 m in Fig. 4. The water column was vertically almost homogeneous until early May. There was no significant change between 19 January and 16 February. During April phosphate concentrations fell sharply, and on 10 May were low throughout the water column. It is reasonable to assume that the vertical mixing possible before the establishment of the thermocline had carried part of the phytoplankton crop below the photosynthetic zone. After 19 May when the thermocline restricted vertical movement of water, this part of the phytoplankton would be trapped at depths where it could not long survive for lack of light. The consequence is interesting. Above the thermocline phosphate remained low (minimum 0·07μg atom P/l. on 11 August), but at deeper levels it increased, presumably by regeneration from the dying phytoplankton.

By 11 October, when the water had become isothermal, phosphate also became uniform, and increased until the end of the year.

SILICATE

The vertical distribution of silicate is shown in Fig. 3 and values at 10 and 50 m in Fig. 4.

On 19 January silicate was 4·7 μg atom Si/l., a little higher than in December 1953, and the highest value recorded here since the present series of observations began in 1950. On 16 February the concentration was 2·8 μg atom Si/l.

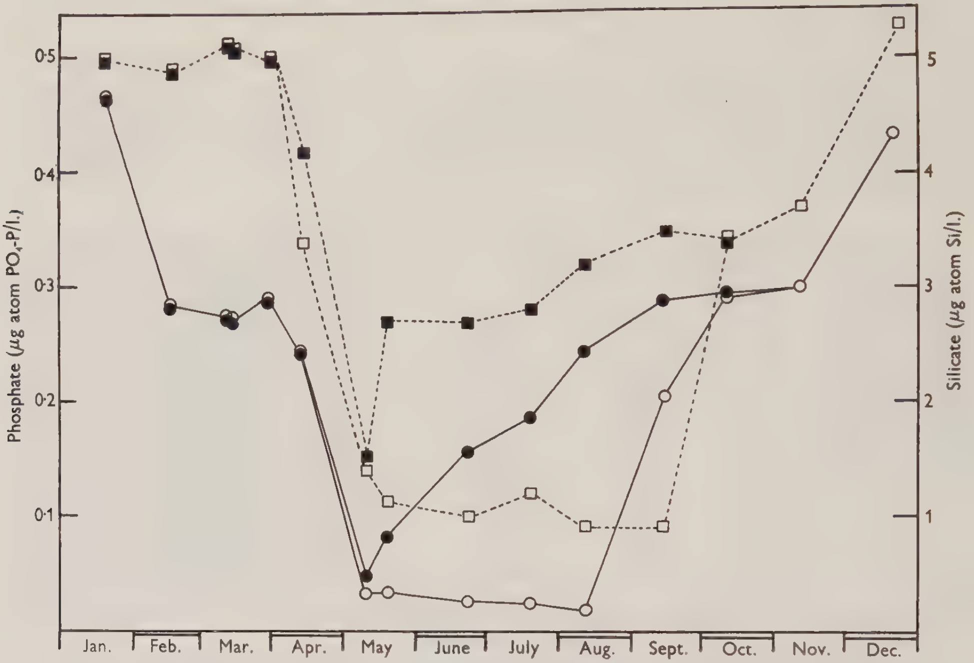

Fig. 4. Phosphate (μg atom PO_4-P/l.) and silicate (μg atom Si/l.) at 10 and 50 m at International Hydrographic Station E1 during 1954. Phosphate at 10 m, □; at 50 m, ■. Silicate at 10 m, ○; at 50 m, ●.

This very large decrease cannot have been caused by growth of diatoms. Such a growth would have been unprecedented at this time of the year, but is ruled out of account since there was no corresponding decrease in phosphate. The change in silicate must be ascribed to an influx of another body of water. There was, as noted above, a small change in salinity.

Silicate values fluctuated somewhat until 12 April, when the mean value was 2·5. On 10 May this had fallen to 0·4 when phosphate had also diminished throughout the water column. After the establishment of the thermocline, silicate remained low in the upper layers (minimum value 0·17 μg atom Si/l. on 11 August) though not so low as in 1953. Below the thermocline there was a steady increase, more gradual than that for phosphate. The values at 50 m in

Fig. 4 show an almost linear increase with time, which suggests that silica was dissolving from the frustules of diatoms trapped and dying below the euphotic zone.

On 14 September silicate had risen to 2·1 µg atom Si/l. in the upper layers, although phosphate remained low. The possibility that diatoms may have been supplanted by non-siliceous algae cannot be excluded, though without more knowledge of the flora at this station this is speculative. It seems certain, considering the marked temperature change and the appreciable alteration in salinity, that there was a change in the water mass. Silicate concentrations increased further in November and December and high values around 4·3 µg atom Si/l. were found in the top 10 m layer on 21 December.

Table II. Integral Mean Concentrations in Water Column at Station E1

Date	Phosphate-P (µg atom P/l.)	'Total-P' (µg atom P/l.)	Silicate (µg atom Si/l.)
19. i. 54	0·50	0·57	4·70
16. ii. 54	0·49	0·61	2·83
12. iii. 54	0·51	0·61	2·74
15. iii. 54	0·51	0·62	2·62
29. iii. 54	0·49	0·62	2·88
12. iv. 54	0·41	0·61	2·47
10. v. 54	0·14	0·40	0·39
19. v. 54	0·19	0·36	0·63
22. vi. 54	0·21	0·37	1·10
19. vii. 54	0·23	0·41	1·32
11. viii. 54	0·24	0·45	1·54
14. ix. 54	0·26	0·44	2·61
11. x. 54	0·34	0·46	2·95
11. xi. 54	0·41*	0·55*	2·94*
21. xii. 54	0·53*	0·64†	3·71†

* Upper 30 m only. † Upper 25 m only.

Integral mean Concentrations

Computed figures are shown in Table II, and need little comment. The decreases representing consumption of nutrients in the spring outburst of plants were: phosphate 0·37µg atom P/l., total phosphorus 0·26µg atom P/l., silicate 2·49µg atom Si/l.

Summary

The results of analyses of water from the International Hydrographic Station E1 during 1954 are discussed. The seasonal variation is shown, in which it appears that consumption of nutrients in the spring outburst of plants was: phosphate 0·37µg atom P/l., total phosphorus 0·26µg atom P/l., silicate 2·49µg atom Si/l., these figures being means for the whole water column. Unusual changes in silicate concentrations between January and February, and between August and September, are ascribed to changes in the water mass at the station. This conclusion is supported by the temperature, salinity and phosphate observations.

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A PELAGIC MARINE DIATOM REQUIRING COBALAMIN

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A bacteria-free culture of the important centric diatom *Skeletonema costatum* has recently been established at Millport, and some exploratory nutritional experiments have been carried out with it. *S. costatum* is a photo-autotroph whose autotrophy is, apparently, limited to cobalamin (vitamin B_{12}).

Details concerning isolation and maintenance of *S. costatum* differ from those already published (Droop, 1954*a*, 1955) only in the matter of pH control, a vital factor in the successful culture of this species whose range of tolerance is narrow (pH 7·5–8·5).

A synthetic solution (*S 36*) was developed for and used in the experiments to be described:

S 36

KNO_3	100 mg	Cobalamin	100 mμg
K_2HPO_4	10 mg	'S.W. 1'*	250 ml.
$Na_2SiO_3 . 9\,H_2O$	100 mg	'S.W. 2'*	5 ml.
Tris-(hydroxymethyl)-aminomethane	500 mg	'T.M. 2'*	10 ml.
Thiamin	1 mg	Glass distilled water	735 ml.

S. costatum has been carried through more than ten transfers in this medium and growth has been as good as in any containing soil and liver extracts and natural sea water. The medium was adjusted to pH 8·1 before being decanted into test-tubes and autoclaved for a few moments at 15 lb., inoculations being carried out 18 hr later from exponentially growing cultures.

The sensitivity of *S. costatum* to pH was such as to require the use of replications in all vitamin experiments. This rendered conventional dose-response layouts very unwieldy. Consequently, the requirement for cobalamin was demonstrated qualitatively by the use of a serial transfer technique in which cultures receiving a full dose of the vitamin were compared with those receiving none. At each stage inoculations were taken from a vitamin-free culture of the previous stage to one batch of medium containing the vitamin and to one vitamin-free, the two batches having been prepared and adjusted as one, then divided for the addition of the vitamin, and autoclaved simultaneously.

The results of these experiments are shown in Table I. The magnitude of the errors occasioned by the difficulty of adequately controlling pH is not sufficient to obscure the fact that significantly poorer yields were obtained in the vitamin-free controls in each trial after the first. Inocula for the latter were from stock, hence carry-over of cobalamin in this instance would not be negligible. The results show that cyanocobalamin, the vitamin proper, can be spared by the variants, pseudo-vitamin B_{12}, factor A and factor B; but the experiments were not such as to be able to determine the relative activities of these variants, nor indeed to determine whether the requirement for cobalamin is absolute.

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* For composition of these metal solutions, see Droop (1955).

TABLE I. EFFECT OF COBALAMIN AT A CONCENTRATION OF 100 $m\mu g$/l. ON GROWTH OF *Skeletonema costatum* ON SERIAL TRANSFER IN MEDIUM *S 36* LESS COBALAMIN

Inocula were taken from controls of previous transfer (dilution factor: 100). Final yields measured optically (per cent absorption).

	Mean percentage absorption	Standard error	Replications
First transfer			
With cyanocobalamin	17	1·6	5
Control	16	0·81	5
Second transfer			
With cyanocobalamin	26	1·1	5
Control	7	0·84	5
Third transfer			
With cyanocobalamin	15	3·8	7
With factor A	13	2·2	7
With factor B	10	2·2	7
With pseudo-B_{12}	8	5·0	7
Control	2	0·62	7
Fourth transfer			
With cyanocobalamin	39	7·5	7
With factor A	44	4·6	7
With factor B	33	9·0	7
With pseudo-B_{12}	37	5·9	7
Control	7	1·9	7

S. costatum resembles *Bacterium coli* in being able to utilize factor B as the sole source of the vitamin, factor B being that part of the molecule, common to the various forms, which remains after removal of the respective nucleotides (Ford & Porter, 1952). The requirement for cobalamin in this instance, therefore, is likely to be occasioned by a synthetic disability concerning this non-nucleotide portion, as in the case of *B. coli* (Ford, Holdsworth & Kon, 1955). It cannot, however, yet be concluded that *S. costatum* is autotrophic with respect to the nucleotide, because the experiments were undertaken with a culture medium containing thiamin; but this may be the case.

A similar series of experiments with thiamin (in the presence of cobalamin) failed to demonstrate a significant requirement for this vitamin, but the use of cotton-wool plugs may account for this.

The culture of *S. costatum* in a state of bacteriological purity has allowed the first insight into the nutritional requirements of an important pelagic organism. It also provides a measure of hope that success will attend efforts to culture other pelagic species so necessary to an understanding of the biochemical ecology of the sea. Indeed, the concept of non-predatory relationships, though more than fifty years old (cf. Lucas, 1938), has, for want of proper experimental material, yet received no detailed support in the field of phytoplankton studies. The need for accessory substances for growth of marine diatoms, first postulated by Allen (1914) and later by Harvey (1939) and others, is paralleled by nutritional deficiencies in investigated euryhaline diatoms and flagellates (Hutner & Provasoli, 1953; Provasoli & Pintner, 1953; Lewin, 1954; Droop, 1954*b*; Sweeney, 1954). Latterly, each investigation has tended to make more probable the general importance of cobalamins in the economy of the sea, though the species investigated have mostly been supra-littoral or estuarine. The requirement in *S. costatum* is, therefore, of interest, and it is noteworthy that in this species, which possibly resembles other pelagic diatoms, there is a lack of specificity in the form of the vitamin preferred.

Grateful acknowledgement is made to Dr L. Provasoli (Haskins Laboratories, New York) for an introduction to the useful pH buffer, tris-(hydroxymethyl)-aminomethane, and to Dr J. E. Ford (N.I.R.D., Shinfield) for samples of factors A and B and pseudo-vitamin B_{12}.

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SOME NEW SUPRA-LITTORAL PROTISTA

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(Text-figs. 1–19)

This communication is devoted to furnishing diagnoses of six organisms recently isolated from supra-littoral rock pools on the island of Cumbrae. Much of their interest derives from their being available in bacteria-free cultures; on the other hand, with one exception, all are frequent and typical members of the brackish supra-littoral.

Accounts of habitats in many ways similar to those on Cumbrae are to be found in Bohlin (1897), Levander (1900), Carter (1937), Conrad (1939) and Droop (1953). According to Bohlin's classification the pools may be divided broadly into three categories: (1) 'brackish', rock pools in which the influence of sea splash is dominant; (2) 'rain', rock pools beyond the normal reach of splash but not influenced by land drainage; (3) 'peat', pools with coloured water receiving drainage from surrounding peaty soil. On Cumbrae pools are found both on the friable Devonian rock and the hard basalt dykes. 'Rain' pools are not plentiful, probably from absence of any agency keeping the rocks beyond the immediate range of sea splash free from phanerogamic vegetation. Where they do occur they usually contain *Haematococcus pluvialis*. The 'brackish' pools are mostly under 10 l. in capacity and are subject to extreme fluctuations in salinity and alkalinity and dry up frequently. Typical dominants other than the species to be described are *Oxyrrhis marina*, *Monochrysis lutheri*, *Chlamydomonas pulsatilla* and *Platymonas* spp. The 'peat' pools, though mostly fresh, are subject to occasional inundations by the sea. Their reaction is normally neutral, as they do not support heavy populations.

CULTIVATION

A modification of Pringsheim's (1946) technique was employed for making isolations (Droop, 1954*a*). Cultures were at first maintained in a complex medium such as medium I below, but later it became possible in a number of cases to substitute for this completely 'synthetic' solutions, of which *S 20* and *S 22* are examples.

Medium I

Soil extract	20 mg[1]	K_2HPO_4	10 mg
Liver extract ('Oxoid')	100 mg	Natural sea water diluted to appropriate strength with glass-distilled water and autoclaved separately from the nutrients	1000 ml.
Tryptone peptone ('Difco')	100 mg		
KNO_3	100 mg		

S 20

KNO_3	100 mg
K_2HPO_4	10 mg
'S.W. 1'	250 ml.
'S.W. 2'	5 ml.
'T.M. 2'	10 ml.
Glass-distilled water	730 ml.

S 22

Glycine	40 mg
Guanine	40 mg
Uric acid	4 mg
Thiamin	1 mg
Cobalamin	100 mμg
Medium *S 20*	1000 ml.

'S.W. 1'

NaCl	60 g
$MgCl_2 \cdot 6H_2O$	10 g
KCl	1·5 g
$CaSO_4$	2 g
Glass-distilled water to	1000 ml.

'S.W. 2'

$SrCl_2$	1000 mg
KBr	3000 mg
$Al_2(SO_4)_3$	50 mg
$LiCl \cdot H_2O$	10 mg
RbCl	10 mg
Glass-distilled water	1000 ml.

'T.M. 2'

EDTA[2]	2000 mg	$NaMoO_4$	40 mg
Fe-EDTA	70 mg	$CoSO_4$	1·7 mg
$ZnSO_4$	560 mg	$CuSO_4$	0·3 mg
$MnSO_4$	200 mg	Glass-distilled water	1000 ml.

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[1] 4 ml. of an extract of soil which yielded 5 g dry wt. of humic substance per l. on acidification.

[2] Disodium ethylene diamine tetra-acetate.

S 20 is a 'synthetic' substitute for enriched half strength sea water (cf. Hutner & Provasoli, 1951; Provasoli & Pintner, 1953). Although possibly needlessly complex, it may be quickly prepared from the stock solutions. It has formed the core of several successful solutions for 'marine' organisms. *S 22* is suitable for *Prymnesium parvum* and *Monochrysis lutheri* and the *Syracosphaera* and *Hemiselmis* described below. The function of the organic ingredients glycine, guanine and uric acid is not at present clear; all appear to be necessary. It is possible that they serve to increase the buffer capacity of the medium in the alkaline region. The growth factors represented may be superfluous in some instances, but a quantitative dose-response relation has been established for the four species mentioned with cobalamin (Droop, 1954*b*), and this vitamin is probably needed by other Chrysophyceae. pH of the medium should be adjusted with Na_2CO_3 to about 8 for most supra-littoral species. Media below pH 7 are toxic and above pH 9 they tend to precipitate unless autoclaving is reduced to an absolute minimum.

CHLOROPHYCEAE

Chlorococcales

Nannochloris oculata n.sp. (Figs. 1–6) (Strain no. 66)[1]

Cellulae globosae 2–4 μ diam., aut solitariae aut aggregatae; chromatophoro singulo, pallido viridi, parietino per parte peripheriam cellulae occupanti; stigmate pallido rubro, rotundo, ad chromatophorum locato; sine pyrenoide, sed 1–3 granis magnis amylaceis parietinis praeditae; tegumento delicatissimo persistanti, sed circum cellulas juvenes inviso et vix maturas apparenti. Propagatur per fissionem pariens cellulas filias duas (non autosporas). Cellulae filiae aut liberes aut ad liminem tegumenti materni dirupti manentes.

Cells globose 2–4 μ diam. solitary or aggregated; chromatophore single, pale green, parietal occupying but a portion of the circumference; stigma pale orange-red, circular, associated with the chromatophore; pyrenoid lacking; starch grains 1–3, conspicuous, parietal; cell-wall very delicate, invisible in young cells, becoming plainer just before division. Cell division into two daughter cells (not autospores). Daughter cells free or remaining at the mouth of the split maternal tegument.

The genus *Nannochloris* Naumann (1931) is taken by Butcher (1952) to include minute *Chlorella*-like organisms without visible cell-wall in which cell-division resulted in two daughter cells. In *Nannochloris oculata* the presence of a delicate external membrane at certain stages of the life cycle casts some doubt on the true affinities of the species. However, since this character varies in its expression, being quite undetectable under some conditions, and at no time sufficiently firm to modify the mode of cell-division characteristic of *Nannochloris*, it was thought most expedient to place the species there. *N. oculata* differs from other described species also in the possession of a pale circular stigma.

The cell-wall is never visible in young cells, which always appear naked. It is seen first in mature cells just before division, when it has the appearance of a faintly granular investment. As division proceeds this investment enlarges so that space is visible between it and the cell. After division, daughter cells are freed by splitting of the investment, but one or both may remain attached to it, leading in extreme cases to a loose subdendroid colony reminiscent of

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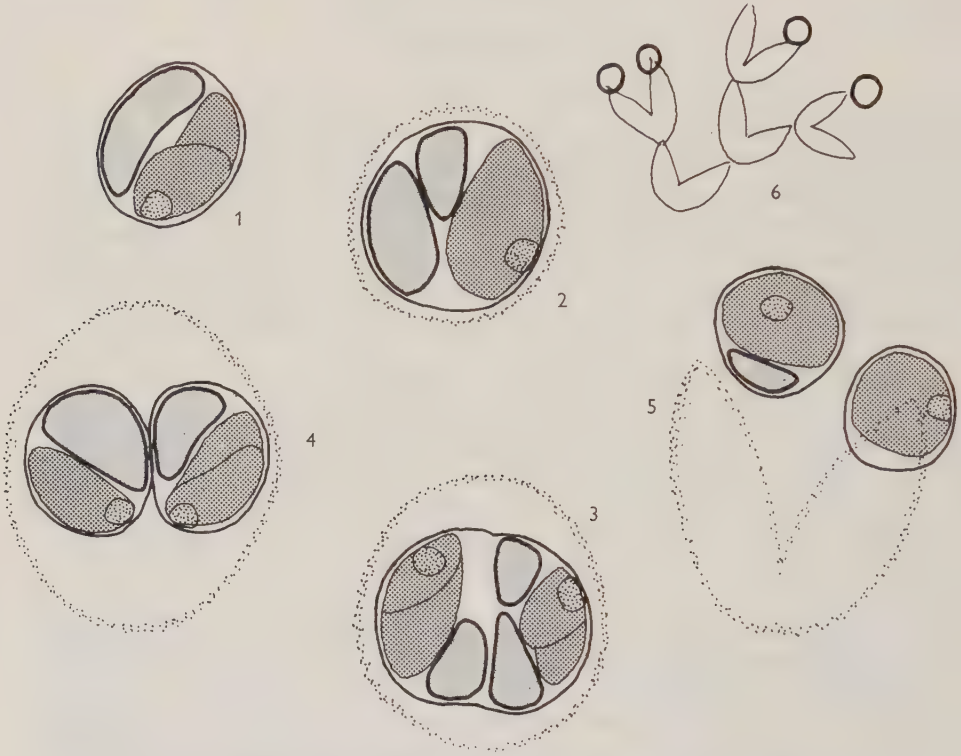
[1] Strain numbers refer to Millport cultures.

Dichotomococcus capitatus Korschikof (1928). The investment stains well with 1 % cresyl blue, when the granulation is very marked. At no time does it appear gelatinous. The environmental conditions leading to its greatest expression are not yet clear to me but I have the impression that they are concomitant with optimum conditions for growth. Colony formation is encountered in the field as well as in cultures.



Figs. 1–6. *Nannochloris oculata* n.sp. Figs. 1–5, stages in cell division, × 7,500. Fig. 6, 'Colony' formation, × 1,750.

The protoplast of *Nannochloris oculata* is clear and structureless and has no visible inclusions other than those mentioned. The chromatophore is pale at all times, but in actively growing cultures is dark enough to make the stigma hard to observe. In nitrogen-deficient cultures, however, it becomes very pale indeed and the stigma is then quite plain. The change in the relative intensity of stigma and chromatophore is reflected in the naked-eye aspect of cultures which turn from yellow-green to brown as if there were carotenoids accumulating.

N. oculata is a frequent inhabitant of brackish rock pools on Cumbrae, particularly those of low salinity on the basalt. Ten out of 115 pools examined in the summer of 1953 contained *N. oculata* as a dominant. Table I shows salinity analysis of these records, including data of the eight species most frequently encountered.

A pure clone (no. 66) of *N. oculata* was obtained by plating a suitable dilution of a sample on 1 % agar with 50 mg/l. soil extract prepared with 50 % natural sea water. Growth was quite good in soil-extract agar but liquid media were improved by casein digest or any of a number of carboxylic acids, purines, and nucleotides, particularly if pH of the medium was on the low side. Medium *S 20*, pH at least 7·5 is satisfactory, but it is more reliable with the addition of 40 mg/l. glutamic acid. *N. oculata* is an obligate phototroph and is non-auxotrophic. It is able to use as N sources nitrate, ammonium, urea, uric acid, and the amino-acid tryptophane. Glutamic acid is also utilized, but very inefficiently (Table II). Tolerance of a wide salinity range in the field indicated in Table I is matched by its performance in cultures in which relative growth rate was found to be maximal (a daily twofold increase with 200 ft candles warm white fluorescent light for 16 h each day) in salinities between 4 and 36 ‰, and cultures could be maintained in salinities between 2 and 54 ‰.

TABLE I. SALINITY DISTRIBUTION OF SOME FIELD RECORDS

(Entries refer to number of records.)

	Salinity (‰)					Total
Species	0 to 0·4	0·4 to 1·6	1·6 to 6·4	6·4 to 25	Above 25	species records
Haematococcus pluvialis	7	1	.	.	.	8
Chlamydomonas pulsatilla	1	3	3	.	.	7
Nannochloris oculata	1	3	3	2	1	10
Euglena proxima	2	2	4	3	.	11
Hemiselmis virescens	.	2	5	4	1	12
Brachiomonas submarina	5	7	15	5	3	35
Platymonas spp.	1	2	1	8	4	16
Syracosphaera elongata	.	1	1	2	2	6
Monochrysis lutheri	.	3	5	7	4	19
Oxyrrhis marina	.	.	7	14	10	31
Total salinity records	12	22	32	23	17	—

TABLE II. UTILIZATION OF SOME N COMPOUNDS IN THE PRESENCE OF SOIL EXTRACT

(+, efficient; ?, doubtful; 0, not utilized; –, compound harmful.)

	Isoleucine	Glutamate	Histidine	Methionine	Tryptophane	Lysine	Leucine	Phenylalanine	Serine	Valine	Glycine	Arginine	Aspartate	Urate	Urea	Ammonium	Nitrate
Haematococcus pluvialis	0	?	?	?	+	?	0	0	0	0	?	+	?	+	+	+	+
Nannochloris oculata	0	?	0	0	+	0	0	0	0	0	0	0	0	+	+	+	+
Monochrysis lutheri	0	0	0	0	–	0	0	0	0	0	+	0	0	+	+	+	+
Syracosphaera elongata	?	0	0	?	–	?	?	?	0	?	?	?	?	+	?	0	+
Prymnesium parvum	+	0	?	?	–	+	?	+	?	+	?	+	+	?	0	?	+

VOLVOCALES

Brachiomonas submarina var. **pulsifera** n.var. (Strains nos. 44 and 45)

Monada speciei *Brachiomonas submarina* Bohlin em. Droop, antice quatuor vacuolis contractilibus praedita.

Cells with the characters of *Brachiomonas submarina* Bohlin em. Droop, but provided with four anterior contractile vacuoles.

B. submarina is a sexual organism with, apparently, two mating types (Droop, 1953); var. *pulsifera* is no exception. Originally twenty-five clones of individuals possessing four contractile vacuoles were isolated, nine of which were of one mating type and 16 of the other. One of each type was retained in culture (strains nos. 44 and 45), and proved to be compatible with one or other of the existing strains of *B. submarina* var. *submarina* (strains nos. 42 and 43). Table III shows the pattern of compatibility.

TABLE III. COMPATIBILITY IN *BRACHIOMONAS*

(Z, compatible; O, incompatible.)

		Strain			
	Strain	42	43	44	45
Var. *submarina*	42	O	Z	Z	O
	43	Z	O	O	Z
Var. *pulsifera*	44	Z	O	O	Z
	45	O	Z	Z	O

Although there has not been undertaken a zygote analysis, which would determine the underlying genetical mechanism, the fact that cells possessing vacuoles can mate with those not possessing them suggests that the difference between the two forms is less than specific. The status Variety seemed appropriate.

Var. *pulsifera* is widespread in brackish pools on Cumbrae. Curiously, the type variety appears to be completely absent.

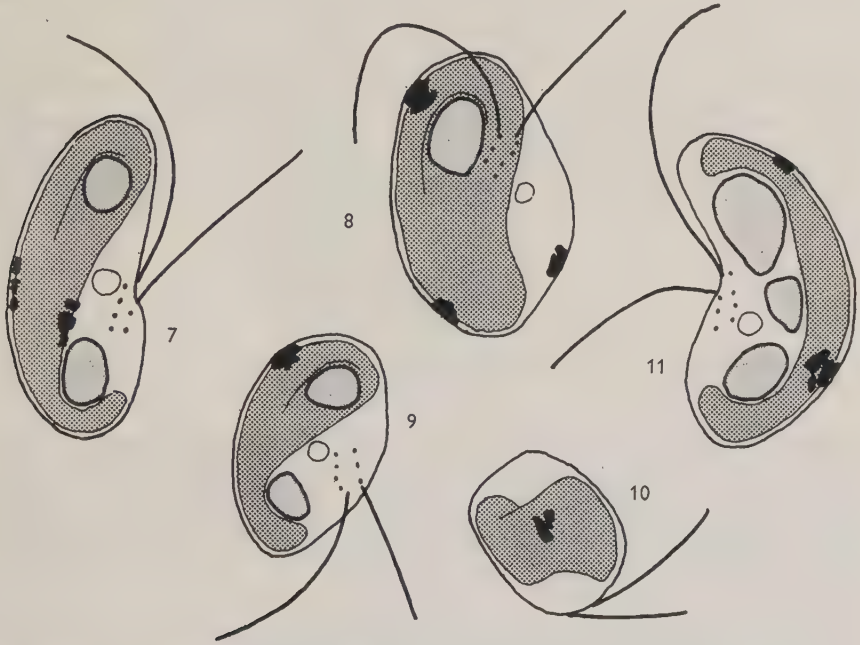
CRYPTOPHYCEAE

NEPHROSELMIDACEAE

Hemiselmis virescens n.sp. (Figs. 7–11) (Strain no. 64)

Monada generis *Hemiselmis* Parke chromatophore laete viride.

Having examined a culture of *H. rufescens* ('Flagellate D' (Parke, 1949)) kindly supplied by Dr Parke, I have been unable to detect any difference between the present species and *H. rufescens* other than colour of the chromatophore. In healthy cultures of *H. virescens* the latter is an almost brilliant green, so that to the naked eye cultures appear pale turquoise to bottle-green according to their density. Figs. 7–11, however, differ from Dr Parke's of *H. rufescens* in the following points: length and stiffness of the flagella, flagellar insertion, number and position of stigmata, and orientation of the two rows of trichocysts. The number and position of the stigmata vary in both species and should in all probability be related to the health of the material. The other differences are, in my opinion, differences in interpretation of small structures. Cells of *H. virescens* are mostly between 5 and 7 μ in length.



Figs. 7–11. *Hemiselmis virescens* n.sp., × 5,000.

H. virescens is widely distributed in supra-littoral pools on Cumbrae; its appearance is rather spasmodic, but on occasions it is to be found in enormous numbers (*c.* 30 million per ml.). Pools of low salinity are favoured but it has a great range (Table I). It is also recorded from Öregrund, Sweden.

The type strain (no. 64) was isolated in July 1953 from a very small pool on Skate Point. The difficulties encountered in isolation of this strain are matched by those of its maintenance. Paradoxically, it has proved easier to maintain in the synthetic medium *S 22* than in any variant of the original isolation medium containing soil and liver extracts. Nutritional requirements are not fully worked out; that room exists for improvement in the medium is shown both by the high yields in nature which cannot yet be achieved in the laboratory and by the improvement frequently obtained in two-member cultures, e.g. cultivated with another flagellate such as *Monochrysis lutheri*. In that case, incidentally, growth of *M. lutheri* is largely suppressed.

The only vitamin requirement of *Hemiselmis virescens* thus far definitely identified is one for cobalamin.

Successful maintenance of this species depends both upon sufficient material (0·2 ml. per 5 ml.) being transferred on subculturing and on cultures not being allowed to age. Subculturing should be carried out fortnightly. Apparently enzymatic changes occur so quickly in cells not actively multiplying that any expression of either 'early' or 'late' lag (cf. Hinshelwood, 1946) is likely to be extreme and to result in complete growth failure. The failure to grow after drastic reduction in cell density may explain why Cryptophyceae are difficult to establish in pure culture by the washing method. The hypothesis that 'early' lag is the time required for the re-establishment of an adequate concentration of a diffusible intermediate in the medium implies that nutritional studies on members of this class will not be complete until that intermediate is identified.

CHRYSOPHYCEAE

COCCOLITHOPHORACEAE

Syracosphaera (Hymenomonas)[1] **elongata** n.sp. (Figs. 12–14)

(Strain no. 62)

Cellulae aut subcordatae aut ovales aut cylindricae antice truncato-rotundatae, postice aut rotundatae aut attenuatae subito in caudam 18–30 × 12–15 μ; coccolithis speciei singularis ellipticis 1·5–2 × 1 μ, absentis ab area instructantis flagellorum; chromatophoris aut duobus aut quatuor; nec stigmatibus neque vacuolis; flagellis cellulae longitudine paratis.

Cells subcordate or cylindrical rounded to truncate at anterior, rounded or suddenly attenuated to a short point at posterior 18–30 × 12–15 μ; coccoliths of a single type, oval, 1·5–2 × 1 μ, absent from area surrounding flagellar insertion; chromatophores 2 or 4; neither stigma nor vacuoles; flagella as long as the cell, not longer. Euryhaline.

S. elongata most nearly resembles *S. carterae*[2] Braarud & Fagerland (1946) particularly as to size, shape and structure of coccoliths. I am indebted to Prof. T. Braarud for electron-microscope observations on the coccoliths of my strain.

The points of difference between *S. elongata* and *S. carterae* which warrant the creation of a new species are size and shape of the cell and length of flagella. *S. elongata* is 1½ times the size of *S. carterae* and the cell length/breadth ratio tends on the whole to be much greater. A ratio of 2–4 is usual. The flagella, on the other hand, are relatively shorter than those of *S. carterae*, being rarely longer and frequently shorter than the cell length. On account of this, *S. elongata* moves noticeably more sluggishly than does *S. carterae*. There is no doubt, however, that the two species are closely related.

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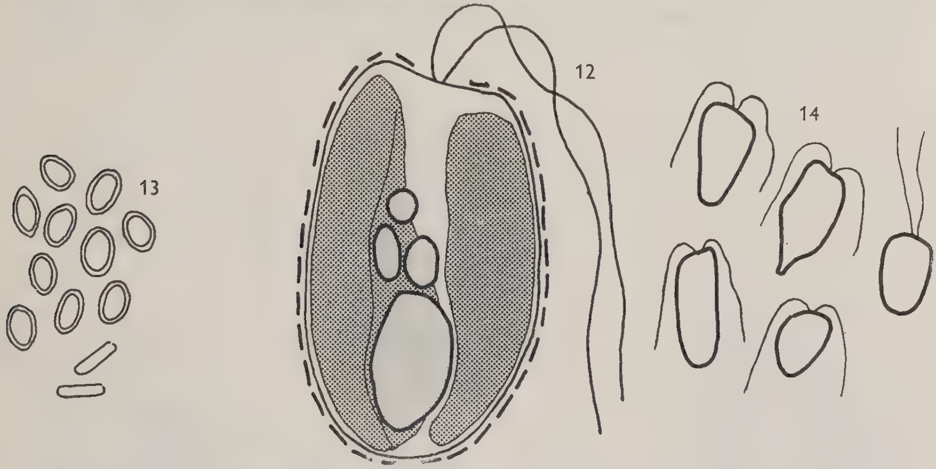
[1] Nomenclature under review (T. Braarud, personal communication.)

[2] Living material of both the Blindern and Plymouth strains has been examined.

S. elongata is a moderately frequent inhabitant of brackish pools of rather high salinity on Cumbrae. Sometimes it occurs in great numbers, though more usually few individuals are encountered.



Figs. 12–14. *Syracosphaera elongata* n.sp. Fig. 12, typical cell in culture, ×2,500. Fig. 13, coccoliths, ×3,500. Fig. 14, cells from wild material, ×500.

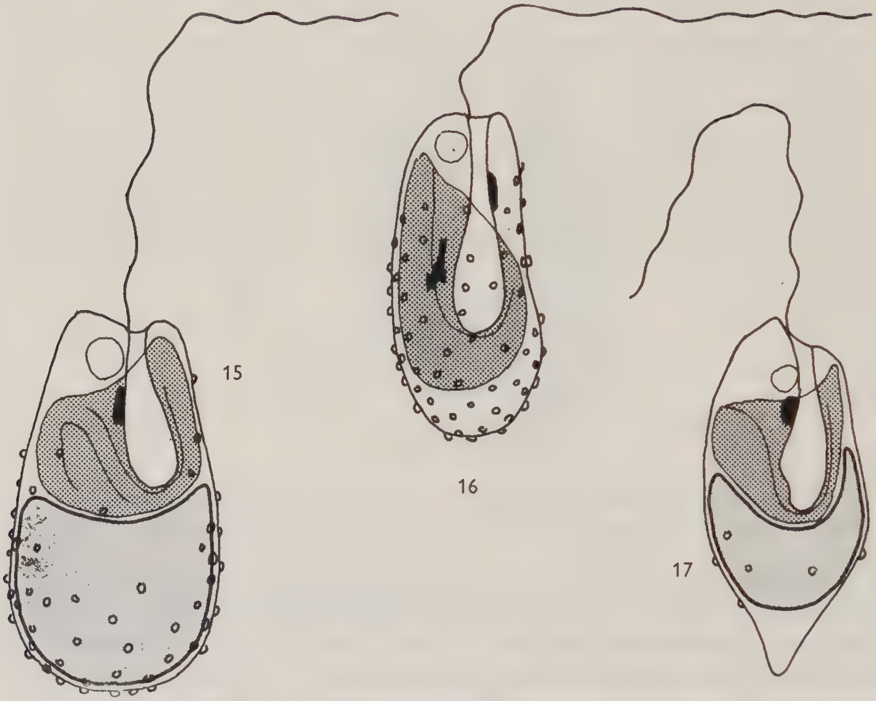
A clone of this species has been maintained for a year in medium *S22*, but has also been cultured in media with salinity as low as 8 and as high as 90‰. There is an absolute requirement for cobalamin and, in certain circumstances, thiamin has a stimulating effect; no other growth requirements have been identified. It appears to be an obligate phototroph, as none of a large range of organic carbon compounds support growth in the dark. Nitrate and uric acid are good sources of nitrogen for this species while ammonium, urea and amino acids are utilized not at all or with difficulty (Table II).

MALLOMONADACEAE

Microglena arenicola n.sp. (Figs. 15–17) (Strain no. 72)

Monada submarina generis *Microglena* Ehrenberg, cylindricato-ovata, paulum asymmetrica et compressa, antice oblique truncato-rotundata depressione vadosa instructa ad oram ampullae, postice aut rotundata aut attenuata subito in caudam, 12–16 μ longae, 8–11 μ crassae; periplasto delicatissimo, antice paucis, postice pluris, siliculis minutis praedito; flagello ad oram ampullae inserto, longiore paulum longitudine cellulae, clinato antice; vacuolo contractili singulo, in ampullam fundenti; chromatophoro singulo aut poculiformi aut sinuato, ventum ampullae circumdato; stigmate magno, laete rubro, ad collum ampullae locato; nucleo postice, sed ante leucosini guttam locato.

Cells cylindrical to ovate slightly compressed and asymmetrical, anteriorly obliquely truncate to rounded with a small shallow depression leading to the reservoir mouth, posteriorly rounded or attenuated suddenly to a point; 12–16 × 8–11 μ; periplast thin with small silicious punctae, mostly at rear; flagellum arising at reservoir mouth, slightly longer than cell, anterior tip inclined sharply during forward movement; contractile vacuole single, emptying into the reservoir; chromatophore single, embracing reservoir belly, cup shaped or folded to an S; stigma large, bright red, at reservoir neck; nucleus lying between chromatophore and posterior leucosin body. Euryhaline.

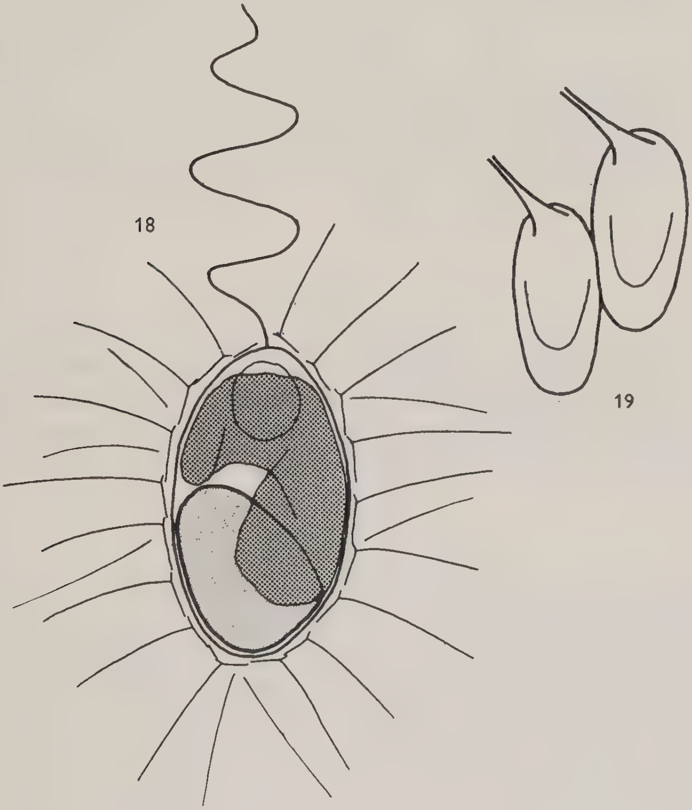


Figs. 15–17. *Microglena arenicola* n.sp., ×5,000.

M. arenicola is similar in shape to *M. punctifera* Ehr., particularly as regards the anterior indention but it is much smaller and relatively narrower anteriorly. Figs. 15 and 16 show typical specimens, while Fig. 17 shows one with the posterior point, possibly associated with exudation of leucosin. The vacuolar apparatus differs from that of other species (cf. Conrad, 1927) in that there is but a single contractile vacuole.

During March and April 1954 high seas had left on the sand of Kames Bay, Millport, a large quantity of brown weed which had become partly buried and rotten. The result was a fine bloom of flagellates and algae on the sand where there was freshwater seepage. Dark green patches were mostly *Euglena proxima*; light green or yellow patches, *Microglena arenicola*. Salinity of the water containing the flagellates was less than 4‰.

No difficulty was encountered in isolating *M. arenicola*, but satisfactory maintenance media await development. At present it is maintained in *S* 22 with or without the addition of silica. It is extremely phototactic in cultures.



Figs. 18, 19. *Mallomonas epithalattia* n.sp. Fig. 18, typical cell, × 1,500. Fig. 19, two plates, × 8,000

Mallomonas epithalattia n.sp. (Figs. 18, 19) (Strain no. 71)

Cellulae ovatae vel ellipsoideae 20–30 × 10–15 μ; scutis ellipticis 4 × 1·5 μ, setis simplicibus aequaliter dispersis et radiantibus 10–15 μ longis; chromatophoro singulo parietino; nucleo antice.

Cells ovoid to ellipsoid 20–30 × 10–15 μ with elliptical plates 4 × 1·5 μ and setae evenly distributed and radially divergent, 10–15 μ long, entire; with single parietal chromatophore and anterior nucleus.

Neither reservoir, contractile vacuoles nor cysts have been observed in *M. epithalattia*. This species differs from others with oval plates in having but a single chromatophore (Conrad, 1933). Except for the shape of the plates, there is a strong resemblance to *M. fresenii* Kent. The plates of *M. epithalattia* are very delicate and are nearly invisible unstained, but they take up aqueous cresyl blue satisfactorily.

M. epithalattia is found in slightly brackish peat pools mainly on Farland Point, Cumbrae. It is more frequent in spring and autumn than in summer. Although samples of water containing this species have been confined to salinities below 3 ‰ it survives and multiplies in cultures with half strength sea water. The isolation medium was soil extract and 1/8 strength sea water with added cobalamin and thiamin. It grows in medium I or in *S 22*, with or without silica, but growth, though repeatable, is exceedingly light. More satisfactory media are being sought.

SUMMARY

Six common supra-littoral Protista from the island of Cumbrae are described and details given of their cultivation. Chlorophyceae: *Nannochloris oculata* n.sp., *Brachiomonas submarina* var. *pulsifera* n.var.; Cryptophyceae: *Hemiselmis virescens* n.sp.; Chrysophyceae: *Syracosphaera elongata* n.sp., *Microglena arenicola* n.sp., *Mallomonas epithalattia* n.sp.

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THE SALP *RITTERIELLA* OFF THE ENGLISH COAST—A CORRECTION

By J. H. Fraser, D.Sc.
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In a recent short note in this *Journal* (Fraser, 1954) the occurrence was announced, off the entrance to the English Channel, of the solitary form of the salp *Ritteriella picteti* (Apstein), and seven aggregate forms of *R. amboinensis* (Apstein), taken by H.M.S. *Challenger* in 1953. The identification of the aggregate forms as *R. amboinensis* and not as *R. picteti* was based, as then stated, on Thompson's (1948) description from a very young specimen of *R. picteti* which emphasized the similarity of its muscle arrangements to those of *Salpa cylindrica* and not to *Ritteriella amboinensis*.

Since this was written, Berner (1954) has described the aggregate form of *R. picteti* from good specimens found off the west coast of U.S.A., and he has shown that the musculature was in fact not like that of *Salpa cylindrica* but very similar to *Ritteriella amboinensis*. Differences were noted in the number of fibres constituting the various body muscles and in the number and arrangement of the oral muscles. A still more recent paper by Yount (1954) includes comparisons of the two forms, based on Berner's characters but also including differences in the length of the endostyle and in body shape (length of anterior and posterior processes, and degree of extension of the gut outside the rest of the body). Yount also goes into greater detail in describing the oral musculature in the two species.

A re-examination of the specimens taken by H.M.S. *Challenger* shows them to be in fairly close agreement with both Berner's and Yount's descriptions of *R. picteti*, but with one or two small differences. The actual number of fibres in the muscles is rather smaller than given by Berner for *R. picteti*, though the precise figure is difficult to ascertain, and there is no median interruption of the first sphincter muscle in the upper lip which Berner believed to be characteristic, and which Yount accepted. I am particularly grateful to Mr Berner for sending me some of his material for comparison and, although I certainly found this interruption in his specimens I do not regard its absence from the Atlantic specimens, or their slightly fewer fibres, as significant differences. Such variations in the musculature are not unexpected, and Sewell (1926), for example, gives a warning (p. 67) against regarding interruptions of muscles, their branching or joining, as diagnostic characters.

Again, the differences in the length of the endostyle and body processes appear to be variable with size, and there is in the Atlantic specimens a range from short blunt processes with the endostyle extending only to M. 5 in the smallest specimen to long filiform processes and extended endostyle in the largest specimens. It thus seems unlikely that these characters can be used diagnostically as Yount suggests.

However, the Atlantic specimens have the four ventral oral muscles as described in detail by Yount and the number of muscle fibres, though less than given by Berner, is considerably greater than that for *R. amboinensis*. In a personal communication Dr H. Thompson tells me that he accepts Berner's description of the aggregate form of *R. picteti* which was based on much more extensive material, and he agrees that his statement (1948) that it has muscle arrangements resembling that of *Salpa cylindrica* can now be ignored. I therefore conclude that the seven aggregate specimens taken by H.M.S. *Challenger* are indeed *Ritteriella picteti* and I wish to correct the original statement. This satisfactorily disposes of the apparent but somewhat surprising coincidence of two most unusual species of salp.

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Contribution No. 1 from the University
of Florida Field Station.

CERCARIA TURRITELLAE N.SP., A 'HUGE-TAILED' MONOSTOME LARVA FROM *TURRITELLA COMMUNIS* RISSO

By Robert F. Hutton
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(Text-figs. 1 and 2)

Rothschild (1935) reported that Dr M. V. Lebour drew her attention to a 'huge-tailed' monostome which had occurred during 1931 in specimens of *Turritella communis* collected from the Rame Mud, Plymouth. From 1932 to 1934 Rothschild (1935) examined 541 specimens of this gastropod from that locality without finding this larva. During the summer of 1952, the present author examined a number of specimens from the same locality and was also unsuccessful in finding such a larval trematode. However, in November 1952, the monostome described below was found in two of 110 *Turritella* collected from Cawsand Bay, Plymouth Sound.

The author wishes to express his indebtedness to the Director and Staff of the Plymouth Laboratory of the Marine Biological Association for their friendly co-operation and assistance in all phases of this work. The work was carried out while the author was holding a Fulbright Scholarship.

METHODS

Fully developed cercariae were obtained by isolating infected specimens of *T. communis* in jars of sea water and allowing the cercariae to emerge. Rediae and immature cercariae were obtained by crushing the shells of the hosts. Living specimens as well as permanent preparations were studied. Whole mounts were fixed in sublimate, stained with borax-carmine, and mounted in Canada balsam, or fixed in sublimate or formalin, unstained, and mounted in Faure's medium or in Cristalite (E. Gurr). Serial sections (5μ) of several cercariae were stained with Ehrlich's haematoxylin and eosin. The orientation of the objects in the desired position at imbedding was achieved by the method of Péterfi (after Romeis, 1948, p. 99). The incidence of infection was determined by crushing the gastropods and examining the digestive glands and gonads under the dissecting microscope.

Cercaria turritellae n.sp.

Specific diagnosis. The body of this non-swimming, non-oculate, monostome larva is elongate. Cuticular spines were not observed on the body. The digestive system (Fig. 2*f*) consists of the following structures: a mouth, located in the centre of a subterminal sucker; a short prepharynx; a small pharynx (?); and a narrow oesophagus leading into two long intestinal caeca that terminate near the posterior end of the body. The excretory vesicle is of moderate size with two lateral branches, containing large refringent inclusions, extending forward and joining in the mid-line of the body well in back of the oral sucker. Underneath the cuticle of the body wall in fully formed cercariae, numerous cystogenous glands, containing small refringent rods, obscure much of the internal structure. The tail is extremely large, wider and somewhat longer than the body; characteristically, it curves ventrally. On the dorsal side of this structure, two finely spined fin-like ridges (Figs. 1*d* and 2*c*, *d*) extend over more than half of its length. Annulations which appear to be circular muscle fibres uniformly cover the tail. Internally, the tail is composed mainly of large hyaline cells, each of which contains a nucleus (Fig. 1*d*). Measurements of fifteen living specimens in various stages of extension and contraction and slightly compressed between a cover-glass and glass slide are (in microns): body length 108–226 (av. 167); body width 46–124 (85); oral sucker width 24–32 (28); tail length 164–260 (212); tail width 62–128 (95). The redia (Fig. 2*e*) is elongate, without collar or ambulacral processes, and provided with a short intestine and a birth pore which is near the pharynx. Lateral to the pharynx is an undetermined number of cephalic glands. The largest rediae contain fully formed cercariae as well as cercarial embryos; no daughter parthenitae were observed. Measurements of three living specimens in various stages of extension and contraction and slightly compressed between a cover-glass and glass slide are (in microns): body length 596–1190 (av. 893); body width 154–306 (230); pharynx width 26–32 (29). The excretory formula of the redia is 2 (1 + 2).

Host. Turritella communis Risso.

Habitat. Digestive gland and gonad.

Locality. Cawsand Bay, Plymouth Sound.

Incidence of infection. 0·57 % (two out of 350 specimens examined).

Remarks. The large tail of these cercariae appears to be an organ used for flotation because there is never any indication that the animals are able to swim, and, as they are usually suspended in the water with the body hanging below the tail, it seems likely that the hyaline cells of which the tail is mainly composed aid the buoyancy of the cercariae. In addition to the large cells, the tail contains near its base, and also bordering the two dorsally placed fin-like ridges, a number of smaller cells (Fig. 1*d*). These two finely spined ridges begin near the base of the tail and extend posteriorly over slightly more than

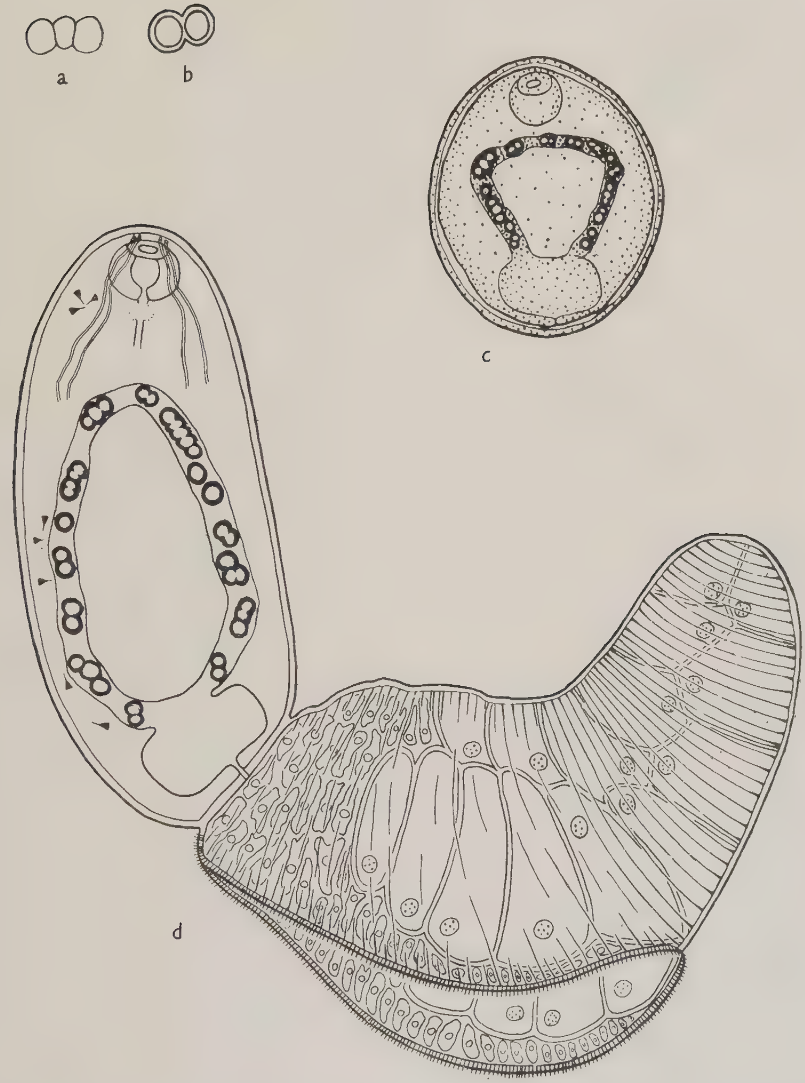

Fig. 1. *Cercaria turritellae.* *a*, thin-walled inclusions in the branches of the excretory vesicle; *b*, thick-walled inclusions in the branches of the excretory vesicle; *c*, encysted cercaria as it appears after sudden pressure from a cover-glass; *d*, fully developed cercaria as it appears under slight pressure from a cover-glass.

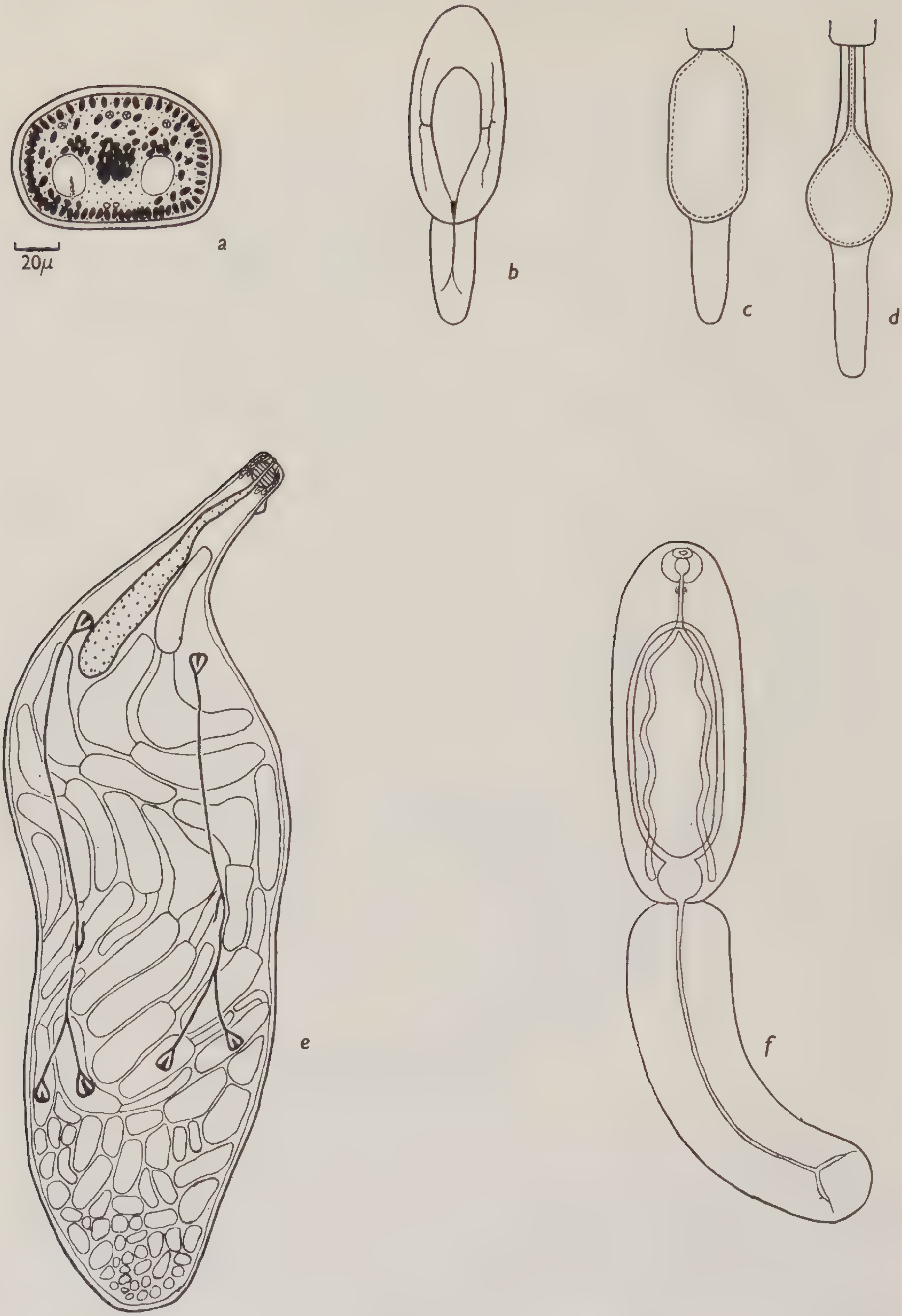

Fig. 2. *Cercaria turritellae.* *a*, transverse section (5 μ) through the body of a fully developed cercaria at a level slightly anterior to the excretory vesicle; *b*, view from ventral side of an immature cercaria showing the type branching of the excretory system in the body and in the tail; *c*, dorsal view of tail showing fin-like ridges as they appear when the tail is contracted; *d*, dorsal view of tail showing fin-like ridges as they appear when the tail is extended; *e*, mature redia showing germ balls, developing cercariae and the excretory system: *f*, immature cercaria showing the digestive tract and part of the excretory system.

half of its length. At that point they curve medially and join together at the mid-dorsal line of the body (Fig. 1 *d*). While the cercariae are suspended in the water, the tails are slowly extended and contracted intermittently. This causes a change in the appearance of the fin-like ridges. Fig. 2*c* shows the shape of the ridges in a specimen with a contracted tail, while Fig. 2*d* is a specimen with an extended tail; both figures are views from the dorsal side.

In laboratory containers, immediately after being released from the molluscan host, members of this species rise in the water and float just below the surface film. Later, if the water remains quiet, the cercariae gradually settle to the bottom of the containers. Slight agitation of the water causes the larvae to rise and float for some time after the agitation stops.

A short time after being released, the cercariae begin to secrete a substance which forms large cocoon-like cysts enclosing the larvae completely. These cysts are composed of a thin substance that is translucent except for numerous scattered wart-like knobs which are composed of small rods similar to, but smaller than, those present within the cystogenous glands of the fully-formed cercaria. While within these cysts, the bodies and attached tails continue to extend and contract intermittently.

Sudden pressure of a cover-glass on unencysted cercariae just released from their molluscan host causes decaudation and encystment. These cysts (Fig. 1 *c*) are approximately five to six times smaller than the cocoon-like structures previously mentioned. Cysts of ten living specimens produced in this manner measure 118–136 μ in length and 94–108 μ in width.

As was stated previously, there are underneath the cuticle of the body numerous cystogenous glands containing a number of refringent rods. The largest of these rods measure 12 μ in length and almost 2 μ in width. They are evidently cystogenous granules similar to those reported by Sinitsin (1911) and Sewell (1922), as well as by other writers. The cuticle of the body is pierced by numerous small openings, each of which appears to lead into a cystogenous gland.

In living fully formed cercariae well extended under pressure from a cover-glass, a structure evidently representing a small pharynx (Fig. 1 *d*) is visible. Only in immature larvae (Fig. 2*f*), in which the cystogenous glands are not so well developed, are the digestive caeca distinguishable. They extend to the posterior end of the body and terminate lateral to the excretory vesicle.

On each side of the oesophagus, a pair of small ducts run forward and open to the exterior in front of the subterminal sucker. Although in serial sections the penetration glands could not be discerned, it seems likely that because of their position these ducts are penetration gland canals. Serial sections (5 μ) of several specimens stained with Ehrlich's haematoxylin and eosin reveal six ducts in addition to those of the lateral branches of the excretory vesicle; however, I was unable to follow their ultimate courses.

Large rounded inclusions within the branches of the excretory vesicle are very conspicuous structures of mature cercariae. They do not occur in the vesicle itself and in the immature larvae are altogether absent. Usually they exhibit characteristic thick walls (Fig. 1*b*), but thin walls sometimes occur (Fig. 1*a*). Figs. 1*c*, *d* show the thick-walled inclusions as they appear under the dissecting microscope using incident light.

A special effort was made to study the details of the excretory system, but the refringent rods underneath the cuticle of the body obscured the pattern of the excretory system of the mature cercaria; however, there is a possibility that the flame cells occur in groups of three since such a group was observed near the oral sucker (Fig. 1*d*). Immature cercariae revealed the type-branching of the excretory system as shown in Fig. 2*b*. From each lateral branch of the vesicle, a single collecting tubule extends for a short distance before bifurcating into anterior and posterior branches. From the posterior end of the vesicle, a single median tubule passes through the tail and bifurcates as shown in Fig. 2*b*, *f*.

Of the two gastropods infected with *Cercaria turritellae* one was a male 29 mm. long while the other was a female 35·5 mm. long.

Discussion

Sinitsin (1911) described a binoculate monostome, *C. equitator*, with a huge tail. This species was described from *Cerithiolum exille* collected from the Black Sea. Miller (1925*a*) described a similar binoculate monostome, *Cercaria purpuracauda*. It was found in the digestive gland of *Bittium eschrichtii* collected from Puget Sound; later (1925*b* and 1929) he described very briefly four additional species (*Cercaria* F, *Cercaria* T, *Cercaria* U, *Cercaria* W) with eye-spots collected from the Dry Tortugas area. Cable (1952)[1] also reported a magnacercous monostome cercaria with eye-spots from *Turritella exoleata* collected from the marine waters of Puerto Rico. This cercaria, like those reported by Sinitsin and Miller, is an active swimmer. Cable believes this larva, from morphological and ecological observations, is a heterophid trematode belonging to the subfamily Galactosominae.

Cercaria turritellae n.sp. differs conspicuously from the above species by lacking eye-spots, by not being an active swimmer, by having a smaller proportion of tail length to body length (with the possible exception of *Cercaria* W since Miller did not give any measurements for this species) and by having fin-like ridges on the proximal part of the tail. Also, this cercaria differs from *C. equitator*, *C. purpuracauda* and the species reported by Cable by not having spines at the anterior end of the body. Large rounded inclusions which occur in the branches of the excretory vesicle of *C. turritellae* are very conspicuous in the fully formed larva; they have not been reported for other marine 'huge-tailed' monostome larvae.

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[1] And personal communication.

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THE FUNCTIONAL MORPHOLOGY OF THE ROCK-BORING LAMELLIBRANCH *PETRICOLA PHOLADIFORMIS* LAMARCK

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(Text-figs. 1–16)

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INTRODUCTION

While the writer was engaged in a study of the British species of Pholadidae, an opportunity arose also to study *Petricola pholadiformis* Lamarck (see Winckworth, 1932), a rock-boring lamellibranch belonging to the Petricolidae. This species is of special interest as it is considered to exhibit convergent evolution with respect to *Barnea candida*, one of the British species of Pholadidae.

The specimens were obtained from Burnham-on-Crouch, Essex, where they were collected near low-water mark by Mr G. D. Waugh, to whom the writer wishes to express his gratitude. Living and preserved specimens of *Petricola pholadiformis* were studied in 1950 at the Department of Zoology and Comparative Anatomy, University College of South Wales and Monmouthshire, Cardiff.

The most important paper on *Petricola* is that of Lamy (1923), who reviewed the genus, describing *P. pholadiformis* Lamarck, and giving its synonyms. He described the distribution of this species on the Atlantic Coast of North America, and on the West Coast of Africa. He recorded its arrival in European waters, where it was observed on the coast of Kent in 1893. The genus is briefly mentioned by Sikes (1910), Calman (1919), Schlesch (1932), Calman & Crawford (1936), and Coe (1943). Other direct references to *Petricola* will be referred to in the appropriate places; wider references to literature are made in a paper on the British Pholadidae (Purchon, 1955).

THE SIPHONS

The basal half of the siphonal process is formed by fusion of the inhalant and exhalant siphons (Fig. 4). The siphons are separate for their distal halves, the inhalant being slightly longer than the exhalant siphon (Morse, 1919). When fully extended, the siphons are a little longer than the shell valves. The siphons are formed solely from the inner lobes of the mantle margin, and are unprotected by periostracum. The middle and outer lobes of the mantle form a crescentic ridge passing round the base of the siphonal process, parallel to the edge of the shell (Figs. 4, 9, *Olm*), and giving rise to a thin transparent sheet of periostracum which passes to the margin of the shell valves. Thus the siphons are of type 'A' as defined by Yonge (1948).

The siphons of *P. pholadiformis* are smooth and the flesh is of an opaque creamy colour with irregular patches of light brown pigment distally.

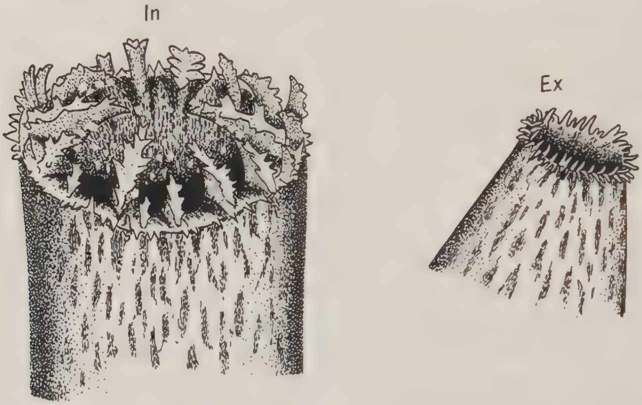

Fig. 1. *P. pholadiformis*, free-hand drawing of the tips of the siphons. Shell length 1·5 in. ×7·5.

The inhalant aperture is surrounded by a series of irregularly pinnate tentacles of various sizes (Fig. 1, *In*). These may be roughly classified as primary, secondary, tertiary and even quaternary according to size, the quaternary tentacles lying closest to the outer margin and being smallest and simplest. There was much variation from specimen to specimen in the sample studied, but there tended to be about six primary tentacles which normally projected across the inhalant aperture. There were about as many smaller secondary tentacles and, external to these, a larger number of still smaller and simpler tertiary tentacles. At the periphery, external to the twenty-four or more tertiary tentacles, there was a ring of about forty-eight small marginal papillae.

The primary, secondary and tertiary pinnate tentacles are very similar in form and pigmentation to the pinnate tentacles of *Zirphaea crispata* (see

Purchon, 1955). In addition, however, in *Petricola pholadiformis* there are occasional opaque white patches on the sides of the tentacles and on the inner surface of the inhalant siphon.

Unlike the inhalant siphon, the exhalant siphon tapers slightly towards its extremity which is similarly ornamented by irregular brown patches (Fig. 1, *Ex*). The exhalant aperture is surrounded by a variable number of small conical tentacles which tend to be arranged in two circles, the inner consisting of about twenty-four tentacles, some of which may be united at their bases in pairs. The outer ring consists of a larger number of smaller tentacles.

When observed in aquaria, the siphons were seldom extended far, the base of the siphonal process generally being partly introverted between the posterior ends of the shell valves. They undergo slight contractions and expansions which are possibly more pronounced under natural conditions.

The Shell Valves

The shell of *P. pholadiformis* superficially resembles that of *Barnea candida* (see Purchon, 1955) and the two species may be found burrowing side by side. *Petricola* may be readily distinguished, however, by the absence of accessory shell plates and by the presence of a conspicuous external ligament. Internally the differences between *Petricola* and *Barnea* are equally pronounced.

The hinge teeth are well developed (Figs. 2, 3, *Ht*) and these, together with the elongated external ligament (Fig. 2, *L*) will undoubtedly prevent any rocking of the shell valves about a vertical axis, as has evolved in the Adesmacea. The adductor scars are in the typical positions ventral to the hinge line, the anterior adductor scar being only a little less in area than that of the posterior adductor muscle.

In the shell drawn, the pallial sinus penetrated to 60% of the total shell length, a value of the same order as has been found in the Pholadidae (Purchon, 1955); *Petricola pholadiformis* is certainly well adapted for burrowing, if not for boring. There is no 'accessory ventral adductor' in *P. pholadiformis* (as is present in the Adesmacea), but there is an 'accessory dorsal adductor muscle', dorsal to the rectum (Fig. 14, *Apa*).

The habit of the specimens here studied has been described by Mr G. D. Waugh (personal communication) as boring in lumps of chalk which lie on the mud at low-water mark. In some cases specimens had bored through the chalk and their anterior ends had entered the underlying mud. *Petricola* is apparently only able to bore into very soft rocks, perforation being presumably by opening and closing of the shell valves on the hinge line as an axis. This view was held by Otter (1937) for *P. lapicida*, the burrow of which he described as oval in section.

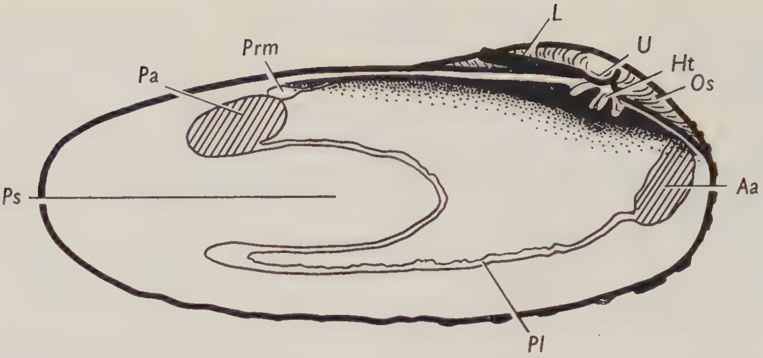

Fig. 2. *P. pholadiformis*, interior of the left shell valve. Shell length 2·3 in. × 1·5. For lettering see pp. 277–8.

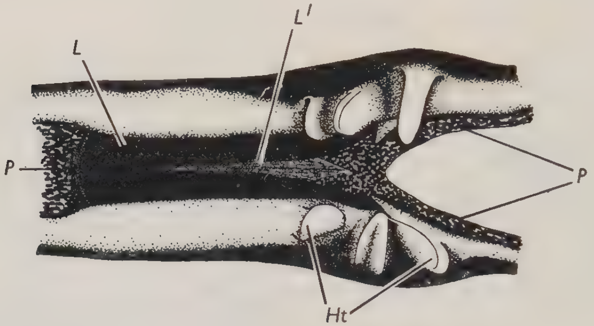

Fig. 3. *P. pholadiformis*. Ventral view of a portion of the hinge line of two shell valves, the valves being widely parted. The anterior end is to the right. Shell length 1·7 in. × 5·3. For lettering see pp. 277–8.

The Mantle Cavity

The disposition of the organs in the mantle cavity is shown in Fig. 4. Detailed description is not necessary. The most important features of the various organs will be outlined in the following sections.

The pedal gape is long, and amounts to about half of the length of the shell (Fig. 4). There is an annular backwardly directed valve in the bases of both the inhalant and the exhalant siphons (Fig. 4, *V*). According to Haas (1929–40) such valves occur in many families. Similar valves are present in *Pholadidea loscombiana* (see Purchon, 1955).

The Ctenida

The outer demibranch (Fig. 4, *Alod*) does not reach as far forwards as the inner demibranch, much of which is therefore exposed to the action of the sorting areas of the outer labial palp (*Rolp*). Particles carried forwards either

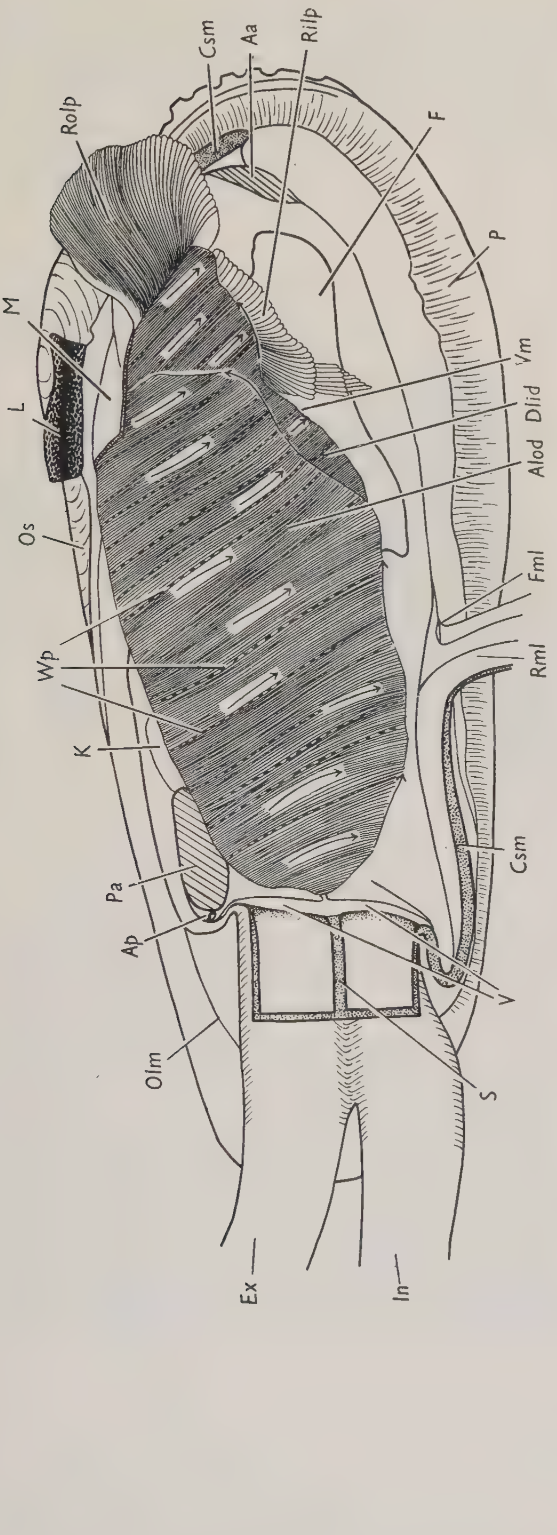
Csm
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Fig. 4. *P. pholadiformis*. Organs in the mantle cavity. The right shell valve and most of the right mantle lobe have been removed. Shell length 1·9 in. ×3. For lettering see pp. 277–8.

in the ctenidial axis or in the marginal groove of the outer demibranch do not travel forwards to the mouth in a distal oral groove anterior to the inner demibranch, but are passed ventralwards over the anterior part of the inner demibranch where they are subjected to selection by the outer labial palp.

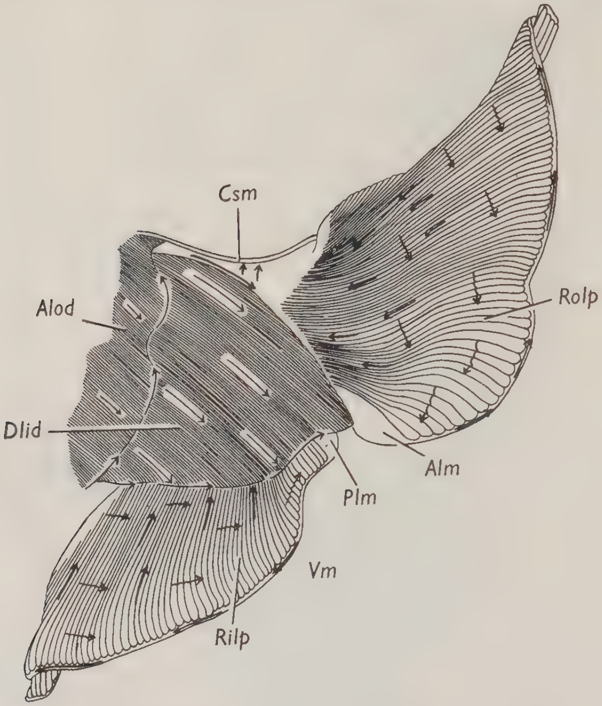

Fig. 5. *P. pholadiformis.* The labial palps of the right side. Shell length 1·9 in. × 5·25. For lettering see pp. 277–8.

Ciliary currents (shown in Fig. 6) were studied by the application of carborundum *F 3* in minute quantities. The ctenidia fall into Atkins's Category C (1*c*) (Atkins, 1937*b*) and are similar to those of *Barnea candida* (Atkins, 1937*a*). They differ from one another in the absence of a marginal groove in the outer demibranch of *B. candida* and in the presence of weak plication in *Petricola pholadiformis* (Atkins, 1937*b*).

Atkins (1937*a*) described similarities between *P. pholadiformis* and various species of the Pholadidae. Thus in this species and in *Pholadidea loscombiana* the marginal grooves are protected by fan-shaped groups of guarding cilia which arch over the groove. On the other hand, cirrus-like cilia are present along the posterior sides of the filaments of *Petricola pholadiformis* and both *Barnea parva* and *B. candida*. These cirrus-like cilia may aid in the removal of large particles of sand or rock which fall on the ctenidia.

There are a number of points in which *Petricola pholadiformis* resembles *Barnea candida*, the most remarkable of which is the presence of opposing ciliary currents on the filaments of the descending lamella of the outer demibranch (Fig. 6). The two species live side by side occasionally, e.g. at Burnham-on-Crouch, and the similarity between their ctenidia may reasonably be regarded as parallel adaptation to similar environmental conditions. This has been discussed by Purchon (1955).

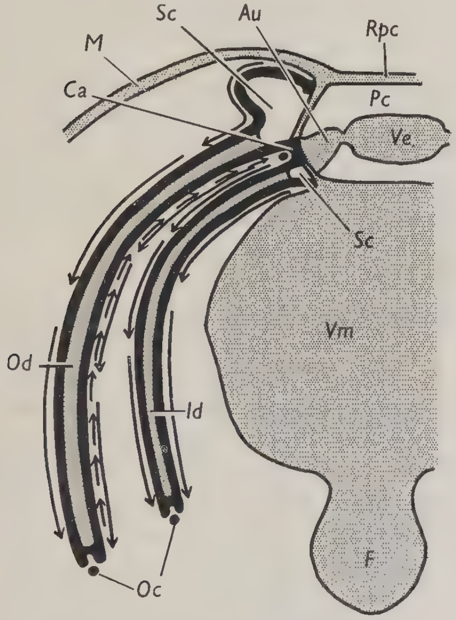

Fig. 6.

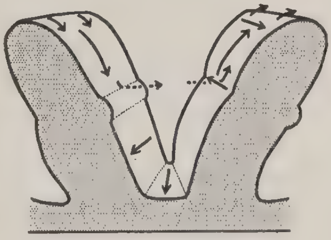

Fig. 7.

Fig. 6. *P. pholadiformis*, a thick hand-cut transverse section through the body in the region of the pericardium. × 10.

Fig. 7. *P. pholadiformis*. Diagrammatic representation of the ciliary pattern on the folds of the labial palps. Anterior side to the right.

For lettering see pp. 277–8.

The Labial Palps

The labial palps are extremely active, and are continually coiling and uncoiling as is indicated in Fig. 4, *Rilp*. The ridges on the opposed surfaces of the palps may be thrown violently into zigzags on the slightest mechanical stimulation of the palps.

The detailed ciliary pattern on the folds of the palps is shown in Fig. 7. The following ciliary currents may be recognized:

(1) *A rejection current* on the floor and sides of the groove between adjacent folds on the palp. Particles are driven to the free ventral border of the palp.

(2) *An acceptance current* operating on the superficial slopes and on the crests of the folds, carrying particles forwards from fold to fold, towards the mouth.

(3) *A ventralward re-sorting current* on the crest of each fold, carrying particles towards the ventral border of the palp.

(4) *A dorsalward re-sorting current* carrying particles in the superficial part of each groove, towards the base of the palp and the lateral oral groove.

In addition, and not shown in Fig. 7, is a rejection tract which conveys material from the grooves backwards to the tip of the palp along its free ventral border. This material is then transferred to the mantle.

Skeins of particles bound in mucus which were passed to the palp from the anterior end of the inner demibranch were not affected by the dorsalward re-sorting currents, since these were obscured by the overlapping of adjacent folds. Such material was rapidly transported anteriorly and ventralwards under the combined action of the acceptance current and the ventralward re-sorting current on the crests of the folds, and was always rejected via the free ventral margin of the palp.

The inner demibranch of the ctenidium (Fig. 4, *Dlid*) passes deeply between the palps, and under normal conditions particles moving over that part of the inner demibranch will be subjected to selection by the folds of the palps. When the palps were presented with suspensions of carborundum *F 3*, particles were predominantly cast rapidly forwards and downwards over the folds, travelling progressively over the folds without being deflected dorsalwards or ventralwards by the re-sorting currents. Such action was never to be seen in the Pholadidae (Purchon, 1955). Sooner or later, however, the particles were trapped in the superficial part of the grooves, and were transported dorsalwards due to the action of the dorsalward re-sorting current. These particles accumulated at the base of the palp, and together with any particles brought thither by the ctenidial axis, passed into the lateral oral groove and thence rapidly to the mouth where they were ingested. Very little entered the deepest part of the grooves to be rejected via the free ventral border of the palp.

In the specimens studied, there was no suggestion of intermittent feeding, as was noted in the Pholadidae (Purchon, 1955), and it was concluded that the palps were far less selective than those of the Pholadidae.

It appears that the palps accept fine and medium-sized particles and convey them to the mouth, but reject mucus-bound material such as is likely to be presented to the palps when the water contains much suspended material.

On the outer smooth surfaces of the palps the cilia beat dorsalwards, and particles were carried over the postero-dorsal free margin of the palps and on to the ridged inner surfaces.

The Visceral Mass and the Foot

The foot is plough-shaped, and lies along the whole ventral border of the visceral mass (Fig. 4, *F*). A broad, apparently unciliated area on the side of the visceral mass (Fig. 8, *Ia*) corresponds in shape and position with that of the inner labial palp, while no ciliary activity could be detected on the sides of the foot. Over the postero-dorsal half of the visceral mass, the ciliary currents carried particles ventralwards, and into posteriorward rejection tracts which carried all material on the sides of the visceral mass to its posterior end. Particles falling on the sides of the visceral mass will presumably be cleared by the labial palps.

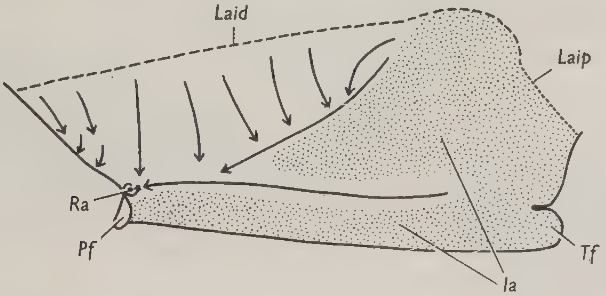

Fig. 8. *P. pholadiformis*. Ciliary currents on the right side of the visceral mass. Shell length 2·3 in. ×2·7. For lettering see pp. 277–8.

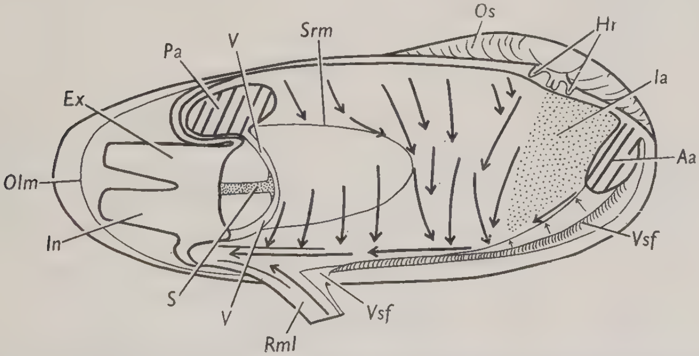

Fig. 9. *P. pholadiformis*. Ciliary currents on the inner surface of the left lobe of the mantle. Shell length 1·9 in. ×1·35. For lettering see pp. 277–8.

The Mantle

In order to study the ciliation of the inner surface of the mantle, the visceral mass and ctenidia were removed and the preparation was left for some hours to allow the muscles to relax and the siphonal process to expand somewhat. There is a broad triangular area anteriorly, which is apparently unciliated and which corresponds in shape and position with that of the outer labial palp (Fig. 9, *Ia*).

Over the greater part of the mantle the ciliary action is ventralwards, carrying particles towards the margin of the mantle. There is a powerful rejection tract near the mantle margin. This arises near the mouth, passes backwards on the side of the pedal gape, and joins with its partner from the other side of the body posterior to the pedal gape. Under experimental conditions, waste material collects in a small pocket at the base of the siphonal process; it is not certain whether this would or would not occur under natural conditions.

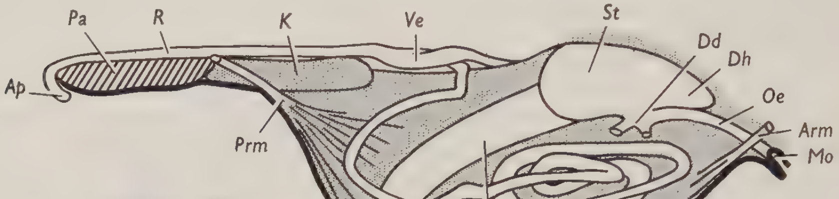

Fig. 11

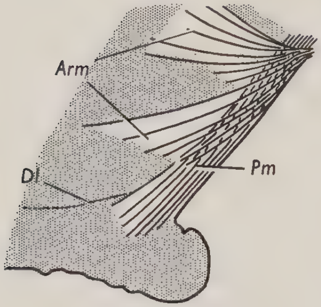

Fig. 10

Fig. 10. *P. pholadiformis.* Muscles serving the anterior part of the visceral mass and the foot. × 5·3.

Fig. 11. *P. pholadiformis.* General dissection of the alimentary canal. Shell length 1·9 in. × 2·7.

For lettering see pp. 277–8.

MUSCULATURE

Unlike the Pholadidae (Purchon, 1955), the anterior adductor scar of *Petricola pholadiformis* is smaller in area than that of the posterior adductor, being 70% of the area in the shell drawn (Fig. 2). Another difference is the position of the anterior adductor scar ventral to the hinge line. There is an 'Accessory Posterior Adductor Muscle', which lies anterior to the posterior adductor and *dorsal* to the rectum (Fig. 14, *Apa*).

The anterior retractor muscle of the visceral mass. This arises as a flattened sheet a little posterior to the anterior adductor (Fig. 10, *Arm*). Its fibres spread out immediately below the epidermis on the antero-dorsal surface of the visceral mass.

The posterior retractor muscle of the visceral mass. This arises anterior to the posterior adductor (Figs. 2, 11, *Prm*), and it is more powerfully developed than that of the Pholadidae. At the postero-dorsal angle of the visceral mass the left and right muscles meet and a proportion of fibres cross over from side to side.

Pedal retractor muscle. This arises immediately behind the anterior adductor, and internal to the base of the anterior retractor of the visceral mass; it is a stout bundle, elliptical in section (Fig. 10, *Pm*). The muscle passes downwards along the anterior face of the visceral mass and as it approaches the muscular tissues of the foot it spreads out posteriorly, and there is a mingling of fibres from left and right sides as the two muscles enter the foot.

Transverse muscle fibres in the visceral mass. Here and there, transverse muscle fibres arise in the epithelium of the visceral mass and pass inwards either to the walls of the alimentary canal or transversely across the visceral mass to the opposite side. No such muscle bundles were found in the Pholadidae, though a few muscle fibres were found in *Zirphaea crispata* inserted into the sides of the oesophagus and the anterior face of the stomach (Purchon, 1955).

The Alimentary Canal

A general dissection of the alimentary canal was carried out on specimens that had been relaxed with menthol and magnesium sulphate, and had then been preserved in alcohol (Fig. 11). The oesophagus enters the ventral wall of the stomach and its base is overlapped by an outgrowth of the stomach, the dorsal hood (Fig. 11, *Dh*). The apertures from the stomach into the digestive diverticula (*Dd*) lie on the ventral surface of the stomach. The combined style sac and mid-gut, the cavities of which are incompletely separated by a typhlosole, arise on the postero-ventral surface of the stomach and pass backwards and downwards towards the heel of the foot. The mid-gut passes from the distal end of this wider tube, coils on the ventral side of the stomach and then passes backwards and upwards to the pericardium. The hind-gut penetrates the ventricle, passes ventral to the accessory posterior adductor (Fig. 14, *Apa*), as noted in the previous section, and terminates in an anal papilla in the typical manner.

The internal structure of the stomach was studied on living specimens. The epithelium covering the visceral mass was removed and the stomach exposed by scraping away gonadial tissues, and by loosening the mass of digestive diverticula. A mid-dorsal incision was made in the stomach wall, and the right side of the stomach was drawn downwards. The dorsal hood was opened, giving a view comparable to that shown in Fig. 12, in which the oesophageal aperture is hidden from view by the anterior part of the gastric shield (*Gs*).

The dorsal hood arches forwards above the oesophagus and opens into the stomach anteriorly on its right side (*Odh*). Its ventral wall is covered with a finely ridged sorting area (Fig. 12, *Sa*). Here cilia beat transversely over the crests of the folds towards the apex of the hood on the left-hand side (*Dh'*), whereas in the grooves the cilia beat backwards and finally reject material via the mouth of the hood (*Odh*) into the intestinal groove (*Gi*).

The roof of the dorsal hood is smooth, and ciliary currents on it pass material backwards, over the right side of the stomach and into the mid-gut. The posterior wall of the dorsal hood bears a minor sorting area (*Sa'*) which passes out of the mouth of the dorsal hood, and across the anterior face of the stomach below the oesophageal aperture (Fig. 13, *Sa'*, *Sa''*). It probably serves to carry particles from the anterior face of the stomach into the dorsal hood.

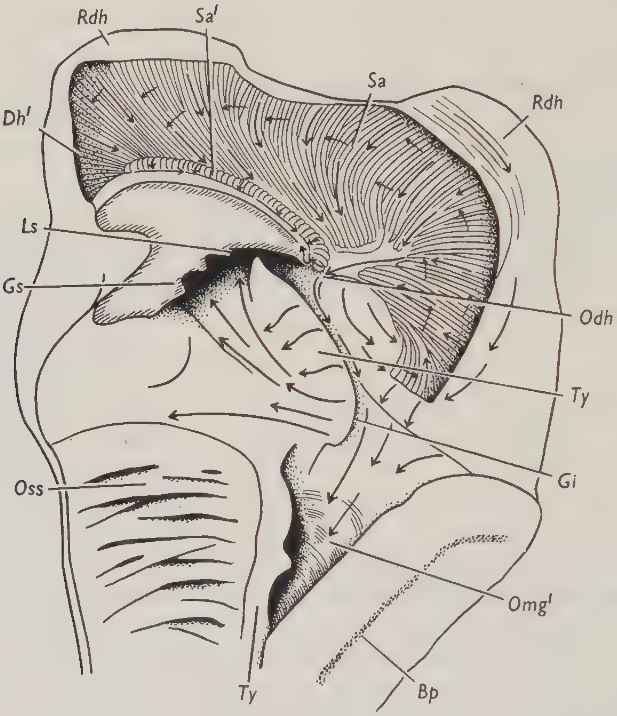

Fig. 12. *P. pholadiformis*. Interior of the stomach. The stomach has been opened by a mid-dorsal incision through the roof of the stomach and style sac, and through the roof of the dorsal hood. Shell length 1·9 in. ×9. For lettering see pp. 277–8.

The left pouch of *Petricola pholadiformis* can be exposed by cutting transversely to the left through the gastric shield (Fig. 12, *Gs*). The sides and floor of the left pouch can now be seen, as also the aperture of the oesophagus into the stomach (Fig. 13). Reference to this figure shows that there is a slender belt of transverse ridges on the roof of the left pouch, there is an extensive sorting area (*Sa'''*) on the floor of the left pouch, and there are also three ducts leading from the left pouch into the digestive diverticula (*Ddd*). It is important to note that the ciliary currents detected in the left pouch do not assist particles to pass into the ducts of the digestive diverticula—on the contrary, the reverse is true.

The major typhlosole (Fig. 13, *Ty′*) arises in the left caecum (*Ddl*), crosses the anterior face of the stomach ventral to the oesophageal aperture and enters the mouth of the right caecum (*Ddr*). It emerges from the right caecum and travels posteriorly along the right side of the floor of the stomach (*Ty*), accompanied all the way by the intestinal groove (*Gi*) and passes into the mid-gut (Fig. 12, *Omg′*).

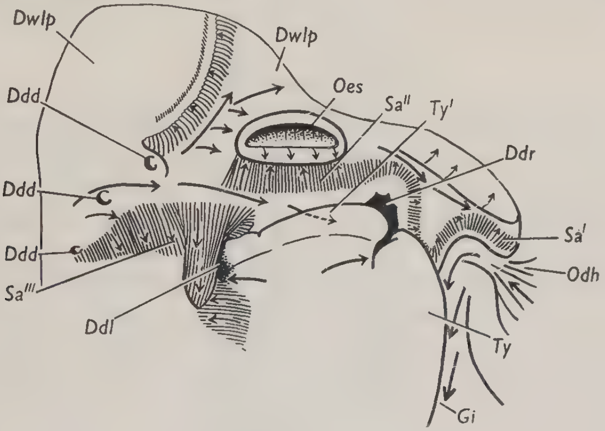

Fig. 13. *P. pholadiformis.* A dorsal view of the anterior part of the stomach, the roof of the left pouch having been cut and turned forwards. × 8. For lettering see pp. 277–8.

The sorting areas described above, in the left pouch, on the anterior face of the stomach, and in the dorsal hood, appear to be expressly designed to hinder the passage of particles from the stomach into the ducts of the digestive diverticula, and to reject any particles captured into the intestinal groove. This applies as much to the ducts of the digestive diverticula which open via the left caecum and the right caecum, as to the ducts that open into the stomach via the left pouch.

Within the mouths of the left and right caeca, the cilia beat inwards, and will carry particles into the ducts of the digestive diverticula. There is no definite ciliary route carrying particles to the left caecum, but particles rejected from the left caecum via the intestinal groove are carried into the right caecum. The intestinal groove finally removes particles rejected by the right caecum, and passes them into the mid-gut.

It appears probable therefore that, as previously noted for the Pholadidae, food material cannot be passed from the stomach into the digestive diverticula solely by ciliary activity. Probably muscular activity is also involved. Changes in the relative volumes of the stomach and the various parts of the digestive diverticula, their ducts and the left and right caeca, could cause stomach contents to be sucked or injected into the digestive diverticula.

Such volumetric changes might be caused by action of the adductor muscles, and by movements of the foot by the pedal muscles. The walls of the stomach may also contribute to movement of the stomach contents. Considerable muscular activity was noted in the wall of the living stomach of *P. pholadiformis*.

It has not been possible to study the action of the crystalline style in the stomach of *P. pholadiformis*. Where the style sac is not completely separated from the lumen of the mid-gut, as in this species, the crystalline style readily dissolves when the animal is prevented from feeding (Yonge, 1926).

THE PERICARDIUM

The heart was studied in living specimens. The pericardial glands, which may either lie on the walls of the auricles, or on the pericardial walls, are here pericardial (White, 1942), as they are in *Choristodon* (*Petricola*) *lapicidum* (see Pelseneer, 1911). The auricles (Fig. 14, *Au*) are triangular with thin walls and the auriculo-ventricular valve (*Avv*) is conspicuous by its opacity and size. As in *Choristodon* (Pelseneer, 1911), there is a large aortic bulb (*Ab*) on the posterior aorta, the contractions of which were studied on freshly captured specimens, at various temperatures. Very great care had to be exercised in the dissection since any leakage of blood soon caused the aortic bulb to collapse permanently.

In dissected specimens the aortic bulb remained inactive as long as the siphons remained withdrawn, but a short time after the work of dissection was completed the siphons were usually protruded slightly and the aortic bulb then became active. After some time, due probably to the gradual loss of blood, the activity of the aortic bulb decreased, and it diminished in size. In Fig. 14 the ventricle is drawn in a contracted state, and the aortic bulb in a moderately expanded state.

By subjecting animals to variations in water temperature, from about 6° C to about 30° C, the rate of beat of the ventricle was found to increase with increasing temperature, but the rate of beat of the aortic bulb was unrelated to temperature. Each beat of the aortic bulb was related to movements of the siphons, which were not related to change in temperature.

The aortic bulb evidently acts both as a safety valve, protecting the ventricle from the effects of a sudden flow of blood from the siphons along the posterior aorta, and also as a reservoir which can quickly supply blood for the subsequent expansion of the siphons. Siphonal contraction and expansion involve considerable movements of blood, and the value of an aortic bulb to a bivalve with very active siphons is obvious.

Specimens of *Scrobicularia plana* were dissected for comparison. This species, which feeds on bottom deposits by means of its inhalant siphon, has very long, separate, and active siphons (Yonge, 1949). As was expected, a large and active aortic bulb was found on the posterior aorta in this species. An animal such as *Petricola pholadiformis*, living in a burrow, and possessing siphons that are united at their bases, may not have to execute very extensive contractions of the siphons in order to bring them within the protection of the burrow. The retention of an active aortic bulb, therefore, may perhaps be taken to indicate that this form has recently taken to a rock-boring mode of life.

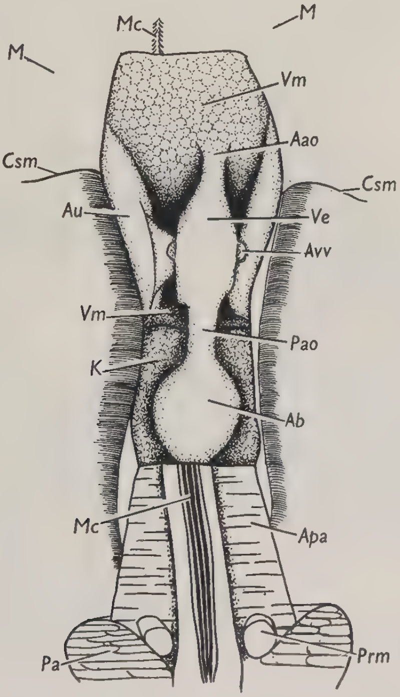

Fig. 14. *P. pholadiformis.* Dorsal view of the contents of the pericardium. Shell length 1·9 in. ×4·5. For lettering see pp. 277–8.

In the Pholadidae, on the other hand, the slightest contraction of the tips of the siphons carries them into the protection of the burrow. Frequent and extensive contractions of the siphons are less likely to occur, and it is not surprising to find that an aortic bulb, if present, is not developed to the same extent as in the case of *P. pholadiformis*. The Pholadidae may be regarded as being more highly adapted to a rock-boring mode of life.

Pelseneer (1906) noted that such highly developed aortic bulbs on the posterior aorta are commonly found in siphonate lamellibranchs. Large aortic bulbs occur in the Veneridae, Petricolidae and Mactridae, for example, and also in the Tridacnidae. Although these last are not siphonate, they probably have similar problems in controlling the movement of blood to and from the mantle whenever the shell valves contract or the mantle margins are withdrawn.

The excretory organ of *P. pholadiformis* (Fig. 14, *K*) lies anterior to the posterior adductor muscle, being overlaid by the aortic bulb which presses slightly into its dorsal surface. It resembles that of the Pholadidae (Purchon, 1955). There is an anterior oval aperture in the median line, causing the cavities of the distal limbs of the left and right excretory organs to communicate. This was found to be the case in *Barnea parva* and in *Zirphaea crispata*, but not in three other species of Pholadidae.

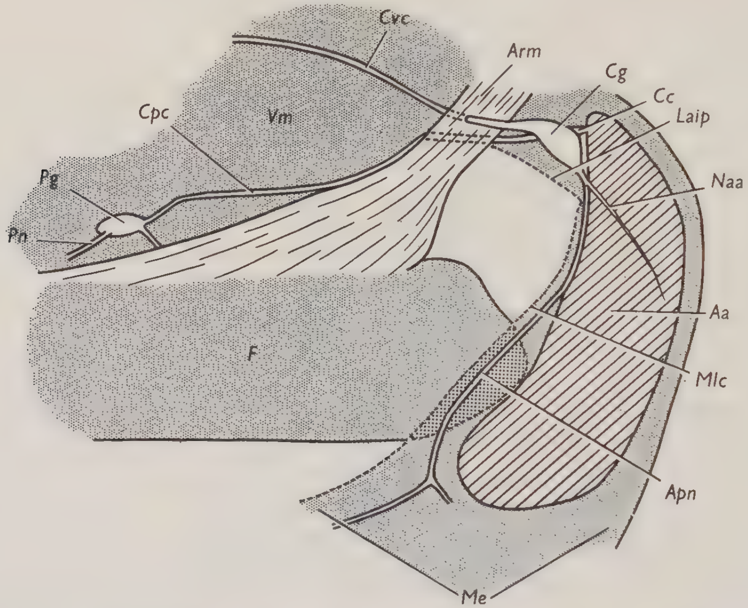

Fig. 15. *P. pholadiformis*. General dissection of the anterior part of the nervous system. Seen from the right side. Shell length 1·8 in. ×9. For lettering see pp. 277–8.

The Nervous System

The cerebral ganglia are of simple fusiform shape and they lie embedded in a tough fibrous matrix, anterior to the anterior retractor muscle of the visceral mass (Fig. 15, *Cg*). The cerebral commissure (*Cc*) passes transversely, close to the postero-dorsal margin of the anterior adductor muscle. The cerebro-pedal connective (*Cpc*) passes backwards internal to the base of the anterior retractor muscle of the visceral mass (*Arm*) and enters the anterior face of the pedal ganglia. The cerebro-visceral connective penetrates the base of the anterior retractor muscle and then passes backwards within the substance of the visceral mass, not close to the epithelium.

The pedal ganglia are large and rounded, and can easily be dissected free from the surrounding tissues. Two large pedal nerves pass out ventrally into the muscles of the foot.

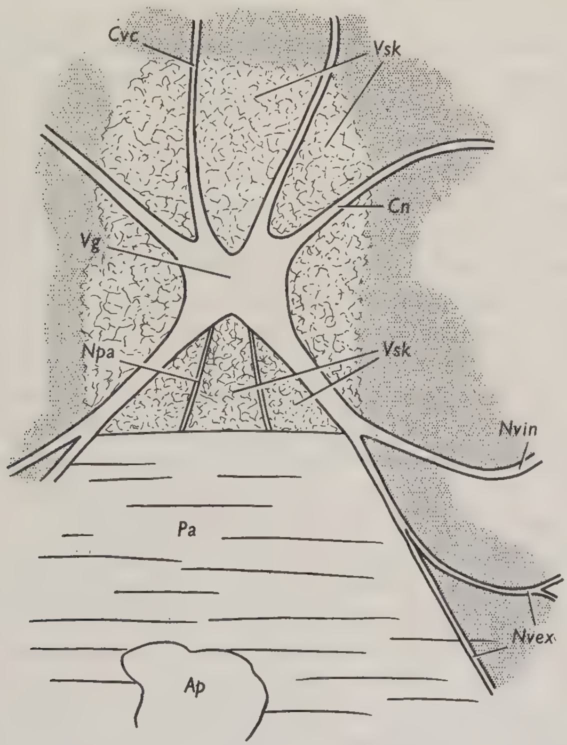

Fig. 16. *P. pholadiformis.* Ventral view of the posterior part of the nervous system. Shell length 1·9 in. ×9. For lettering see pp. 277–8.

The visceral ganglia lie, not on the ventral surface of the posterior adductor muscle, but a little further forward on the ventral surface of the excretory organ (Fig. 16, *Vg*). The yellowish ganglia are easily cleared from surrounding tissues. There is no transverse connective between the cerebro-visceral connectives anterior to the visceral ganglia, as is found in the rock-boring Pholadidae (Purchon, 1955). The following nerves leave the visceral ganglia: a ctenidial nerve (*Cn*), a nerve to the posterior adductor muscle (*Npa*) and a nerve to the siphonal process. The last-named nerve divides into two branches, one serving the inhalant siphon (*Nvin*) and one the exhalant siphon (*Nvex*). Each of these subdivides further in the vicinity of the annular valve in the base of the siphon which it serves. There is no macroscopic indication of a siphonal ganglion on the course of the siphonal nerve.

Discussion

The presence of a large external ligament and of well-developed hinge teeth in *Petricola pholadiformis* indicates that the only motion of the shell valves can be by normal opening and closing about the hinge line as an axis, as shown for *P. lapicida* by Otter (1937). This accounts for two other features in which *P. pholadiformis* differs structurally from the Pholadidae: the anterior adductor is smaller, not larger, in transverse section than the posterior adductor muscle, and there is no accessory ventral adductor muscle to secure a ventral point of articulation for the shell valves.

The separation of the inhalant and exhalant siphons, and the ability to introvert the base of the siphonal process, indicate that *P. pholadiformis* does not bore deeply into rocks and is not highly adapted for rock-boring.

Ciliary mechanisms on the ctenidia and labial palps confirm that the species is adapted for life on sheltered, muddy shores rather than on open rocky coasts. The ctenidia and labial palps appear likely to accept particulate matter rather indiscriminately, but to reject mucus-bound material. On an open coast turbidity would seldom if ever be high, but turbulence of the water would probably result in suspended particles of various sizes being present. The essential feature of the ciliary feeding mechanism of an organism in such an environment would be to select certain grades of particles and to reject others. In sheltered waters, on the other hand, where extreme turbidity may be frequently encountered, particles are likely to be of small size. In such an environment, a ciliary feeder might well accept particles almost indiscriminately until excess turbidity was reached. Heavy secretion of mucus would then cause rejection of the excessive material, probably without completely stopping the passage of some food particles to the mouth.

The labial palps exhibit far more muscular activity than those of the Pholadidae, and this may be to assist in clearing the infra-branchial chamber of excessive quantities of mucus-bound material.

The stomach of *P. pholadiformis*, though oriented differently, is of the same fundamental plan as that of the Pholadidae, and it functions in the same manner. The only significant anatomical differences are the anterior displacement of the dorsal hood, and the conjoined style sac and mid-gut in *P. pholadiformis*. As noted elsewhere for the Pholadidae (Purchon, 1955), ciliary sorting mechanisms within the stomach appear to collect particles for passage into the mid-gut; they would seem to hinder passage of food particles into the ducts of the digestive diverticula. This may be brought about by a muscular pumping or suction action, by relative changes in the volumes of the stomach and of the digestive diverticula.

The bulbus on the posterior aorta appeared at first sight to be directly related to the ventricle in its mode of action, but this was later found not to be the case. Frequency of ventricular contraction was found to be directly related to the temperature, whereas that of the bulbus was related, not to the water temperature, but to the movements of the siphons. It seems probable that the bulbus acts as a safety valve, protecting the ventricle from sudden return of blood from the contracting siphons. It also probably acts as a reservoir from which blood may flow into the siphons when these are extended again.

P. pholadiformis, as its name implies, has been considered to exhibit some degree of convergent evolution with reference to the Pholadidae, and especially with regard to *Barnea candida* (L.) which it resembles superficially, and with which it may occur side by side on the same beach.

During the present study over thirty anatomical points were noted in which *Petricola pholadiformis* differs from all of the British Pholadidae. There can be no doubt that the Pholadidae and the Petricolidae are unrelated. Nevertheless, there are points of resemblance, no less than fourteen of these being noted in which *P. pholadiformis* resembles one or more species of the Pholadidae. But from the point of view of illustrating convergent evolution through adaptation to a rock-boring mode of life only three of these need to be considered, namely:

(*a*) Fan-shaped groups of guarding cilia arch over and protect the marginal grooves of the ctenidium (compare *Pholadidea loscombiana*).

(*b*) Cirrus-like cilia are present along the posterior sides of the ctenidial filaments (compare *Barnea parva*, *B. candida*).

(*c*) The ciliary currents on the ctenidia are of Atkins's type C (1*c*) (Atkins, 1937*b*) (compare *Barnea candida*). An opinion has been expressed elsewhere (Purchon, 1955) that this ciliary pattern on the ctenidia of *B. candida* and *Petricola pholadiformis* has been achieved by simplification of the ciliary mechanisms on three of the ctenidial lamellae and not by complication of the ciliary mechanisms on the fourth lamella.

It seems probable that there must have been some measure of adaptive radiation in the Pholadidae, and also, doubtless, a certain amount of evolutionary 'drift' in each species. Due to variation in structure from species to species within the Pholadidae, it seems inevitable that there should be fortuitous similarities in structure between *P. pholadiformis* and individual members of the Pholadidae. Though the superficial resemblance is to *Barnea candida*, similarities also exist between *Petricola pholadiformis* and other members of the Pholadidae, but these similarities appear to be fortuitous.

SUMMARY

Living specimens of *Petricola pholadiformis* Lamarck were obtained from Burnham-on-Crouch, Essex.

Studies of the principal organ systems were made on the living animal, particular attention being paid to the ciliary feeding and cleansing mechanisms in the mantle cavity, and the ciliary sorting mechanisms within the stomach. The action of the bulbus on the posterior aorta was also studied.

A detailed comparison was made between *P. pholadiformis* and all the British species of Pholadidae. Thirty anatomical features were found in which *P. pholadiformis* differed from *all* of the Pholadidae, and fourteen features in which *P. pholadiformis* resembled one or more species of Pholadidae. It was concluded that these resemblances were purely fortuitous.

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ABBREVIATIONS USED IN THE FIGURES

Aa	Anterior adductor (muscle, or scar)
Aao	Anterior aorta
Ab	Aortic bulb
Alm	Anterior lip of the mouth
Alod	Ascending lamella of the outer demibranch
Ant	Anterior
Ap	Anal papilla
Apa	Accessory posterior adductor muscle
Apn	Anterior pallial nerve
Arm	Anterior retractor muscle of the visceral mass
Au	Auricle
Avv	Auriculo-ventricular valve
Bp	Brown pigment on the wall of the style sac
Ca	Ctenidial axis
Cc	Cerebral commissure
Cg	Cerebral ganglion
Cn	Ctenidial nerve
Cpc	Cerebro-pedal connective
Csm	Cut surface of the mantle
Cssi	Combined style sac and mid-gut
Cvc	Cerebro-visceral connective
Dd	Openings into the digestive diverticula of the right side
Ddd	Ducts of the digestive diverticula
Ddl	The left caecum, into which open the majority of the ducts of the digestive diverticula on the left side of the body
Ddr	The right caecum, into which open all of the ducts of the digestive diverticula on the right side of the body
Dh	Dorsal hood
Dh′	Distal end of the dorsal hood
Dl	Dividing line between the musculature of the foot and the glandular visceral mass
Dlid	Descending lamella of the inner demibranch
Dwlp	Dorsal wall of the left pouch
Ex	Exhalant aperture
F	Foot
Fml	Anterior limit to the fusion of the mantle lobes
Gi	Intestinal groove
Gs	Gastric shield
Ht	Hinge teeth
Ia	Areas of ciliary inactivity
Id	Inner demibranch
In	Inhalant aperture
K	Excretory organ
L	Ligament
L′	Split in the ligament
Laid	Line of attachment of the inner demibranch
Laip	Line of attachment of the right inner labial palp
Ls	The left pouch of the stomach
M	Mantle
Mc	Median mantle crest
Me	Thickened mantle edge
Mg	Mid-gut
Mlc	Line along which the right mantle lobe has been cut away
Mo	Mouth
Naa	Nerve to the anterior adductor muscle
Npa	Nerve to the posterior adductor muscle
Nvin	Nerve to the muscles and valve of the inhalant siphon
Nvex	Nerve to the muscles and valve of the exhalant siphon
Oc	Oralward current
Od	Outer demibranch
Odh	Opening of the dorsal hood into the stomach
Oe	Oesophagus
Oes	Opening of the oesophagus into the stomach
Omg	Origin of the mid-gut at the base of the style sac
Omg′	Origin of the mid-gut from the stomach
Olm	Outer and middle lobes of the mantle edge
Os	Outer surface of shell
Oss	Opening of the style sac into the stomach
P	Periostracum
Pa	Posterior adductor (muscle, or scar)
Pao	Posterior aorta
Pc	Pericardium
Pf	Posterior end of the foot
Pg	Pedal ganglia
Pl	Pallial line
Plm	Posterior lip of the mouth
Pm	Pedal retractor muscle
Pn	Pedal nerves
Post	Posterior
Prm	Posterior retractor muscle of the visceral mass (muscle, or scar)
Ps	Pallial sinus
R	Rectum
Ra	Region where small particles accumulate

Rilp	Right inner labial palp
Rml	Portion of right mantle lobe, turned downwards
Rdh	Roof of the dorsal hood of the stomach, turned outwards
Rolp	Right outer labial palp
Rpc	Roof of the pericardium
S	Septum in the siphonal process
Sa	Sorting area which extends from the interior of the dorsal hood to the intestinal groove
Sa′	Additional sorting area within the dorsal hood, which passes out towards the oesophagus
Sa″	Extension of *Sa′* which lies ventral to the opening of the oesophagus into the stomach
Sa‴	Sorting area within the left pouch of the stomach
Sc	Supra-branchial chamber
Srm	Line of attachment of the siphonal retractor muscles to the shell
St	Stomach
Tf	Tip of the foot, greatly contracted
Ty	Major intestinal typhlosole
Ty′	The part of the major intestinal typhlosole which passes transversely across the stomach from the aperture of the left caecum to that of the right caecum
U	Umbo
V	Valves in the base of the exhalant and inhalant siphons
Ve	Ventricle
Vg	Visceral ganglia
Vm	Visceral mass
Vsf	Velum surrounding the foot
Vsk	Ventral surface of the excretory organ
Wp	Weak plication of the ctenidia

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CAROTENOID PIGMENTS IN THE OPTIC CUSHION OF *MARTHASTERIAS GLACIALIS* (L.)

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(Text-figs. 1–3)

Knowledge of the pigments of photosensitive structures in invertebrates is scanty; this is especially so in echinoderms. In other groups of animals and in plants, carotenoids have frequently been associated with light-sensitive processes (Wald, 1945), though their precise function is still uncertain.

Observations on the occurrence and distribution of carotenoids in various starfishes have been made by several authors whose work is reviewed by Fox (1953). Those in the integument of *Asterias* have recently been described by Vevers (1952), but, apart from the work of Lönnberg & Hellström (1931), and Lönnberg (1933), there have been no recent studies on the carotenoids of *Marthasterias*.

A study of the carotenoids in the light sensitive areas of echinoderms is therefore highly desirable, and the prominent and accessible 'eye spots' of *M. glacialis* (L.) form an obvious choice for such studies, although their functioning as photoreceptors has not yet been demonstrated experimentally in this species.

Several workers, especially Smith (1937), who reviewed the earlier work, have studied the structure of the 'eye spots' in this animal, but no special attention has been given to their pigments. In view of the possible importance of these pigments in visual physiology, it was decided to examine them in living material.

Distribution of Pigment

Individuals were obtained from trawl hauls taken in 20–30 fathoms off Plymouth and their optic cushions excised and examined under the microscope in sea water either directly, or in the form of frozen sections, in order to determine the distribution of pigment. It is essential to avoid the use of fixatives or processes causing shrinkage (as shown by Fox & Millott, 1954), or denaturation, and of solvents which leach out the pigment. Paraffin sections could not, therefore, be used.

The appearance of the excised optic cushion with its pigments is shown in Fig. 1. The main anatomical and histological features of the organ (as they appear in fixed material), have already been described by Smith (1937) and will be mentioned here only in so far as they differ from previous accounts and are essential to understanding pigment distribution. In general form the optic cushion resembles a saddle astride the base of the azygos tentacle, measuring 1·5 mm in length in an average-sized specimen. It bears about 150 deeply pigmented cups (*o.c.*) disposed regularly in rows. Each cup is orientated with its long axis normal to the surface of the cushion, and most measured some $150\,\mu$ in length.

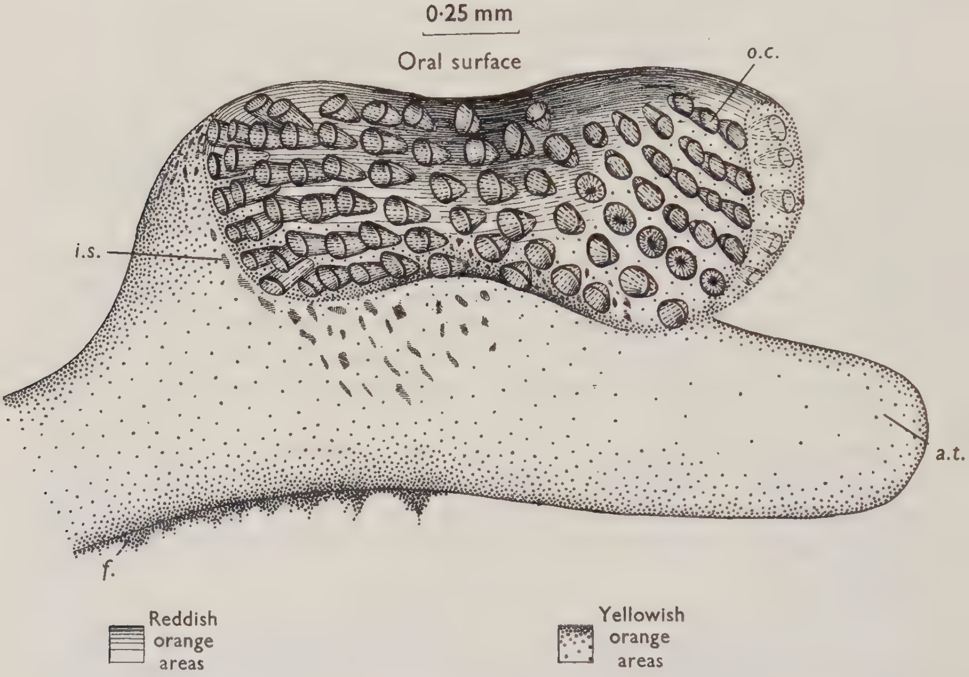

Fig. 1. The optic cushion and azygos tentacle of *Marthasterias glacialis* as it appears when dissected from the arm tip. Semi-diagrammatic, side view, looking obliquely from the tip of the arm. *a.t.*, azygos tentacle; *f.*, fringe marking region where the cushion has been dissected away from the ambulacrum; *i.s.*, one of the scattered, isolated spots of reddish orange pigment; *o.c.*, optic cup.

The cups were called 'optic cups' by Smith, and though their functioning as photoreceptors has not been demonstrated directly, their structure and experimental evidence of the function of similar organs in other starfish justify at least tentative retention of the term.

The cushion and azygos tentacle is yellowish orange, whilst the cups are very conspicuous owing to their deep reddish orange colour. The latter pigment also occurs on the cushion in the form of irregular scattered spots (*i.s.*).

In each cup, as shown in Fig. 2, the pigment is confined to certain cells (*p.c.*) in the wall, which as a result appears brilliant reddish orange in life; the cells at the bottom and around the rim of the cup appear to contain the most pigment. Between the reddish orange cells the wall of the cup appears pale yellow, and in these areas no cellular structure was discernible. It is essential to be cautious in interpreting this appearance, for it might be an artifact, the yellow areas being due to gaps between the deeply pigmented cells, caused by compression of the optic cup by the coverslip during examination. That this is not so is shown by the fact that such areas were observed in fresh cups examined by a water immersion objective and which were not, therefore, subjected to pressure.

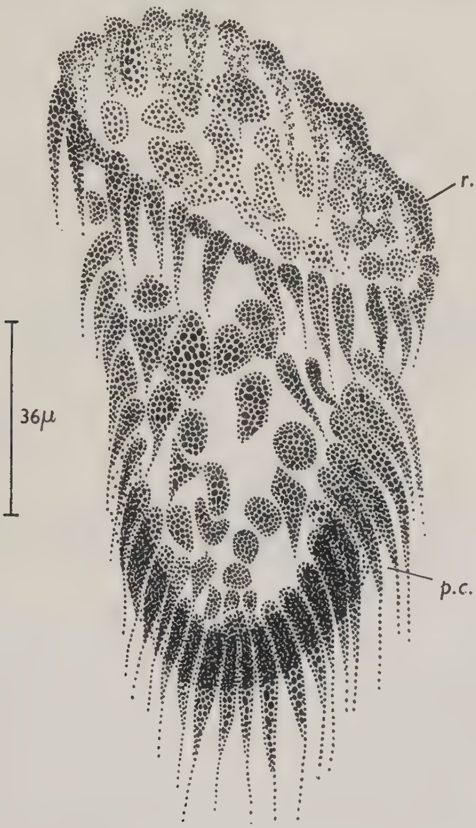

Fig. 2. The distribution of pigment cells in a single optic cup. Drawn from a fresh, unstained optic cushion mounted in sea water. No attempt has been made to show the precise character of cell contents. *r.*, rim of cup; *p.c.*, pigment cell.

Fig. 3 shows the form of the cells bearing the reddish orange pigment. Most are long and narrow with one end drawn out (Fig. 3A); some are irregular in form (Fig. 3C, D) though still attenuated. Within the cell the precise disposition of pigment is difficult to determine. Undoubtedly some is carried in discrete red, or brownish, vacuoles or granules, measuring 0·5–2·0 μ (*gv.*), but more often the pigment is so dense as to prevent accurate observation. Though this can be made out in fresh material, it is more evident in cups which have been left beneath a coverslip for some hours, with the result that some of the pigment disappears (possibly due to oxidation, see p. 283), and observation becomes easier. Even when present in smaller quantity, however, we could not determine the precise way in which some pigment was carried, for it coloured the cytoplasm uniformly yellow or reddish orange, without any discernible association with discrete cellular inclusions (Fig. 3A, C and D).

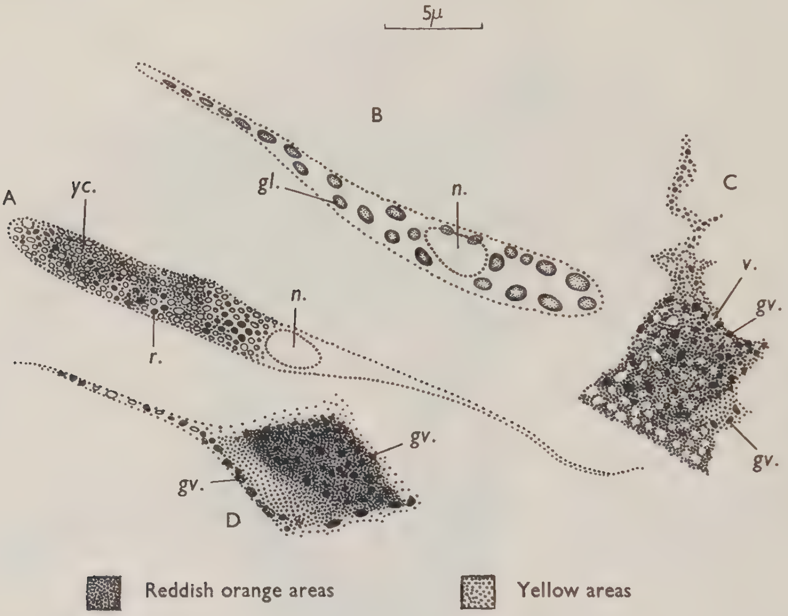

Fig. 3. Isolated pigment cells from the wall of the optic cup, drawn from unstained preparations mounted in sea water. A, fusiform cell from a fresh preparation; B, fusiform cell from an optic cup which had been compressed and left under a coverslip for 36 h, showing the aggregation of pigment to form globules; C, D, irregular cells from fresh preparations. *gl.*, globule of reddish orange pigment; *gv.*, granule (or vacuole) of reddish orange pigment; *n.*, large ovoid inclusion, possibly a nucleus; *r.*, granule (or vacuole) of reddish orange pigment; *v*, colourless inclusion, or vacuole; *yc.*, area of cytoplasm bearing yellowish pigment. The stipple key is applicable only to C and D.

A condition that may possibly be significant was observed in preparations which had been squashed or kept beneath a coverslip for 36 h. In the resultant disorganization, the reddish orange pigment had aggregated into ovoid bodies (Fig. 3B), suggesting that originally the pigment may have been disposed in the form of liquid, or semi-liquid, droplets.

IDENTIFICATION OF THE PIGMENTS

Previous investigations into the pigmentation of asteroids have shown the importance of carotenoids in the general body colour (e.g. Fox, 1953; Vevers, 1952). In *M. glacialis* previous work has suggested the presence of carotenes and xanthophylls (Lönnberg, 1933; Lönnberg & Hellstrom, 1931) and whole extracts of the integument have yielded no evidence of porphyrins, despite the occurrence of protoporphyrin in the integument of the related *Asterias rubens* (Kennedy & Vevers, 1953).

Extracts of the optic cushions of *Marthasterias* were prepared as follows. The optic cushions and azygos tentacles of individuals from the hauls mentioned above were excised intact and freed from adhering integument. Some 200 of these were transferred to refined absolute methanol maintained in the dark in an atmosphere of nitrogen. Extraction was continued for about 12 h to several days at room temperature, and produced an orange solution, leaving behind colourless tissue. It proved essential to conduct the extraction in nitrogen as the solution became colourless in a few hours if left in air.

The crude extract was examined in a Unicam S.P. 500 spectrophotometer for its absorption in the range 320–620 mμ. It showed a broad peak in the region of 472 mμ, suggesting that the colour is due to a mixture of carotenoids.

The pigments were further characterized by two methods.

Method A

Sufficient water was added to the extract to make a concentration of approximately 90% methanol and this was shaken with an equal volume of 'Analar' petroleum ether (B.P. 40–60° C). Nearly all of the pigment passed into the petroleum ether epiphase, leaving only traces of yellow colour in the hypophase of which there was never sufficient for definite characterization. This pale yellow hypophase probably represented small amounts of xanthophylls. The epiphase was slowly evaporated to dryness on a warm bath, then dissolved in a small quantity of ethanol and saponified by warming the solution with 2½ times its volume of KOH (concentration of 160 g KOH in 106 ml water). The resulting saponification mixture was cooled by adding distilled water and then extracted with di-ethyl ether. About two-thirds of the pigment passed immediately into the ether which was collected and evaporated to dryness. This formed fraction *a*1.

The hypophase, which was in aqueous ethanolic KOH, remained in the lower layer when shaken with more ether, and only passed into the epiphase on acidification with glacial acetic acid. This pigment was collected as fraction *a*2.

The two fractions were examined spectrophotometrically and showed the absorption maxima (in mμ) given below:

	Carbon disulphide	*n*-hexane
*a*1	484	450
*a*2	499–500	—

Method B

An extract similar to that used in method A was made and taken by dilution into 'Analar' petroleum ether. This was passed down a column of alumina (B.D.H. for chromatography) and elution was continued with petroleum ether. Two bands formed, one (the lower) being deep orange, the other (upper) pale orange. Development of the chromatogram was continued using 0·5% methanol in petroleum ether, until the two bands were well separated. They were then collected separately, the lower forming fraction *b*1, the upper, fraction *b*2. Both were taken into appropriate solvents for spectrophotometric determination of their absorption maxima which are reported (in mμ) below:

	Carbon disulphide	*n*-Hexane
*b*1	485	449
*b*2	503	—

The phase partition behaviour of fraction *a*1 and the behaviour on the chromatogram of fraction *b*1, together with their absorption maxima, suggest that both are β-carotene.

The absorption maximum of fraction *b*2 and its behaviour when chromatographed on alumina, suggest that it is esterified astaxanthin.

Fraction *a*2 was epiphasic before saponification, but after this it became hypophasic and remained so when in alkaline solution. The absorption maximum of this portion after saponification suggested astacene, and this, together with the original phase partition behaviour, indicates derivation from esterified astaxanthin.

THE EFFECT OF EXPOSURE TO LIGHT

A methanol extract of optic cushions in an atmosphere of nitrogen, contained in a tube of ordinary glass, was irradiated by a 'Hanovia' ultra-violet lamp. Care was taken to place the solution far enough from the lamp to avoid any significant rise in temperature. The optical density of the extract was determined in the region of its maximum absorption in the visible range. Before irradiation log E was found to be 413 at 476 mμ. After 1 h irradiation, it had fallen to 404 at 476 mμ and after a further 2 h, to 276 at the same wavelength. At least one of the pigments is therefore unstable in light.

DISCUSSION

The discovery of β-carotene and astaxanthin in the optic cushions of *Marthasterias* is not surprising in view of their occurrence in the integument of *Asterias* (Vevers, 1952), and since β-carotene has been reported as the predominant carotene in asteroids (Fox, 1953).

It would appear from considerations of molecular size, that the yellowish orange colour is due to β-carotene, while the darker reddish orange is due to esters of astaxanthin. If this is so, from appearances under the microscope, β-carotene is widely distributed in the cushion while astaxanthin appears confined to the optic cup and pigment cells scattered irregularly over the surface of the cushion. Both pigments appear to exist in some of the pigmented cells of the optic cups, although it is not yet clear whether they occur free, or combined, for example, with protein. As regards astaxanthin, combination with protein appears unlikely, since the pigment does not appear in the living optic cushion with the dark hues commonly associated with such a combination, and correlated with this is the fact that the colour of the optic cushion is not markedly changed by a denaturant such as methanol. Some of the reddish orange colour could, however, be due to molecular associations involving β-carotene.

Our findings concerning the appearance of the pigment-bearing cells of the optic cup differ somewhat from those of Smith (1937), who described them as prolonged into tails which are bent so as to lie parallel to the long axis of the optic cup, with their pigment in granular form, confined to the outer third of the cell.

As will be evident from Fig. 2, the form and disposition of the pigment cells leaves no doubt that they correspond to the cells similarly distinguished by Smith, but we find that their abundant pigment is present throughout the cytoplasm. It is also noteworthy that they are about twice the size of those figured by Smith. As previously noted, some of the cells are irregular, though still attenuated.

In general, these differences can be reconciled with the different methods used, for the preparation of paraffin sections by Smith's methods would not only leach out and destroy the carotenoid pigments, but would also cause shrinkage. What the granular pigment mentioned by Smith may be, we cannot suggest, for we saw nothing in the living optic cups that might correspond with it.

As noted by Smith, who reviewed the earlier work, the histology of the optic cup is difficult, but he concluded that it is lined by cells that bear pigment and those that are sensory. A study of the histology of the cup is beyond the scope and methods of this investigation, but we feel it necessary to urge caution in discriminating between sensory and pigmented cells on histological grounds alone. For if the lining of the optic cups is truly photosensory (see below), then since the very nature of the photoreceptive process renders a light absorber necessary, the possession of an orange or yellow pigment absorbing at the high-energy end of the spectrum would seem to constitute an admirable adaptation for this. It is thus possible that the pigment cells are also sensory.

There is general agreement that asteroids are light sensitive, but since the function of the optic cushions in *Marthasterias* has not yet been demonstrated, the role of the carotenoid pigments must remain uncertain. Nevertheless, it must be admitted that in so far as their structure is concerned (see Smith, 1937), the optic cups present evidence of participation in some form of light perception, though due caution must be exercised in using such evidence alone. Furthermore, in the related *Asterias*, the 'eye spot' has been shown unquestionably to be photosensitive by Hartline, Wagner & MacNichol (1952), who recorded its action potential in response to stimulation by light. In addition, however, evidence has accumulated showing that photosensitivity in starfish is not confined to the eye spot and may extend to the tube feet, and, as in other echinoderms, to the general body surface (Cowles, 1910, 1911; v. Frisch, 1909; McCurdy, 1912; Mangold, 1909; Millott, 1955; van Weel, 1935).

The discovery of the same carotenoids in the optic cushion of *Marthasterias* as have been found in the skin of other asteroids, may be significant in possibly providing a clue to the common basis of photosensitivity in these areas, especially since carotenoids have been found in a wide variety of photosensitive structures (Wald, 1945).

If such an indication proves to be well founded, then the optic cushion might well represent a localized concentration of such skin pigments and constitute a stage in the evolution of the condition where photosensitivity is largely confined to highly specialized eyes.

It must be borne in mind, however, that these pigments may not be the only ones occurring in the optic cushion, for the methods of observation and extraction used here would not reveal any fugitive photosensitive pigments of the rhodopsin type, and neither time nor the amount of material available permitted us to determine whether there were other indications of such pigments in the form of vitamin A. There is also the possibility that the pigments may play only a secondary part in light perception by acting as filters, etc. Astaxanthin appears to play such a role in some specialized eyes (Wald, 1945).

Regrettably the experiments made to determine whether the pigments were stable in light could not be completed. Though decomposition occurred, it appears too slow to be significant in vision, but as the pigments were dissolved in methanol they were far removed from their normal chemical environment. Such stability, however, would not in itself prevent the pigments from functioning in photoreception, since their primary function of absorbing light energy would be discharged, whether they remained intact, or were decomposed and reformed.

We wish to thank Dr G. A. Steven and the crews of the Plymouth research vessels for their efforts in obtaining material for us during inclement weather. Our thanks are also due to Mr F. A. J. Armstrong for help and suggestions, and to Prof. J. E. Smith who has kindly read the manuscript. One of us (N.M.) wishes to acknowledge the generosity of the Director and Staff of the Plymouth Laboratory in providing facilities for research.

SUMMARY

The optic cushion of *Marthasterias glacialis* is seen to contain yellowish orange and reddish orange pigments. The former occurs throughout the cushion, the latter is confined to the wall of the optic cups and a few scattered, irregular patches. Both pigments occur in the cytoplasm of long and narrow or irregular cells, some in the form of granules or vacuoles.

Optic cushions yield an orange extract in methanol which decomposes in light.

The behaviour of methanolic extracts after partitioning, saponification, chromatographic separation and spectrophotometric examination indicates the presence of β-carotene and esterified astaxanthin.

The significance of the results in relation to previous findings and their possible bearing on photoreception is discussed.

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A NOTE ON THE RELATIONS OF CERTAIN PARAMETERS FOLLOWING A LOGARITHMIC TRANSFORMATION

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(Text-fig. 1)

The logarithmic transformation has been used in the statistical analysis of certain marine biological data. Parameters calculated from the transformed distributions have been used in the description of the observations, but it appears that some of the mathematical procedures adopted have not been fully understood.

Winsor & Clarke (1940) have analysed data from plankton hauls. The problem considered by them was the determination of the variability in numbers of animals caught by repeated hauls through the same body of water. The raw data were characterized by a constant coefficient of variation, i.e. the variability in catch was proportional to the size of the catch. By transformation from the actual numbers caught to their logarithmic values, it was possible to equalize the variances and to apply the method of analysis of variance to estimate the variability due to the different sources. Finally, an estimate of the coefficient of variation was obtained from the logarithmic values. Their method of estimation was quoted by Snedecor (1946, p. 451) and was employed by Barnes & Bagenal (1951) in their study of repeated trawl hauls. Barnes & Bagenal also calculated confidence limits for comparison of observations following the method of Silliman (1946) in his work on pilchard eggs. These methods seem to be based on a misunderstanding of the nature of a transformation. The formal relation between the variance of such transformed data and the coefficient of variation of the untransformed data follows from the moments of the 'log-normal' distribution, given first by Wicksell (1917). From these moments it will be shown that the method given by Winsor & Clarke is mathematically unsound. However, the discrepancies between their values and those now obtained are comparatively small (ranging from about 2% for a coefficient of variation of 20% to about 15% for one of 60%). In all cases the method given in this paper results in a smaller estimate.

For comparison of catches Silliman gave confidence limits which were expressed as percentages of the mean catch. The mean used, although not explicitly stated, was the geometric mean of the catches.

THE LOGARITHMIC TRANSFORMATION

For untransformed data with a positively skew frequency distribution a transformation from the values of x, to the logarithmic values $y = \log_{10} x$, may result in a distribution of the normal form. The relation between the two distributions is such that the proportion of observations falling within a given range of the x values will equal the proportion of observations falling within the corresponding range of y values. This is so since there is a corresponding value of y to every value of x.

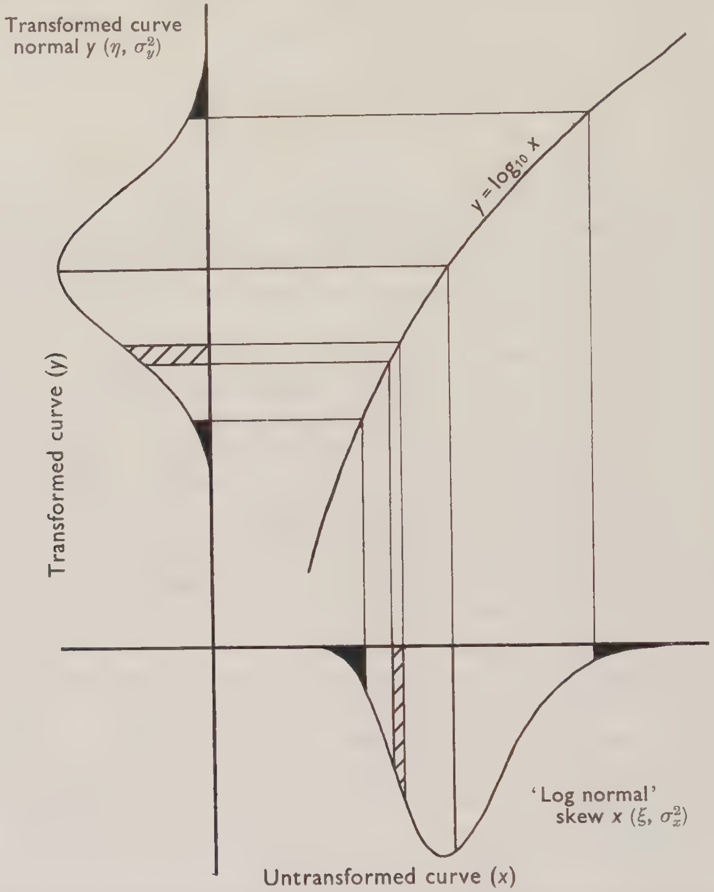
Transformed curve
normal y (η, σ_y^2)
$y = \log_{10} x$
Transformed curve (y)
'Log normal'
skew x (ξ, σ_x^2)
Untransformed curve (x)

Fig. 1. Diagram to show the relation between the 'log-normal' and the normal distributions. For explanation see text.

Such a transformation is shown in Fig. 1 (after N. L. Johnson, 1949).

An untransformed frequency distribution, which is noticeably skew, is represented on the lower margin. Let this be the distribution of x with mean

ξ and variance σ_x^2. In the centre of the figure the relation $y=\log_{10} x$ is represented graphically by the smooth curve. On the left-hand margin is the transformed frequency distribution, projected from the original distribution by means of the curve $y=\log_{10} x$. This distribution of y, with mean η and variance σ_y^2, is normal.

The cross-hatched columns representing frequencies over corresponding ranges of the two variates are equal in area. By consideration of this property of correspondence of areas certain relations between parameters of the two distributions become apparent. The mean, median and mode of the transformed distribution, which is normal, coincide and divide the area under the curve equally. The corresponding value in the untransformed distribution also divides the area under the curve equally and is the median, but because the curve is skew will not coincide with the mean and mode.

The normal curve has been thoroughly investigated and tabulated and use has been made of many of its properties. The 95% confidence limits are those values of the variate between which 95% of the total observations lie, and for the normal curve are approximately given by the mean plus and minus two standard deviations (or for means of small samples t standard deviations). In the figure these are at $\eta \pm 2\sigma_y$ for the transformed distribution, and the corresponding values in the untransformed distribution will also represent 95% confidence limits since the shaded 'tail areas', are equal.

The mean and variance of a frequency distribution do not depend simply on area and therefore the values for one distribution cannot be obtained simply by transformation of the appropriate values for the transformed distribution; but must be obtained from the mathematical relations between them.

Wicksell (1917) gives for the transformation $y=\log_e x$, where y is normally distributed with mean η and variance σ_y^2, the relation:

$$r\text{th moment about origin of } x=e^{r\eta+\frac{1}{2}r^2\sigma_y^2}, \tag{1}$$

whence,

$$\text{mean } x=e^{\eta+\frac{1}{2}\sigma_y^2}, \tag{2}$$

and

$$\text{variance } x=e^{2\eta+\sigma_y^2}\,(e^{\sigma_y^2}-1). \tag{3}$$

For the transformation $y=\log_{10} x$, rearrangement gives the relations

$$\text{mean } y=\eta=\log_{10}\left[\frac{\xi}{(1+\sigma_x^2/\xi^2)^{\frac{1}{2}}}\right], \tag{4}$$

$$\text{variance } y=\sigma_y^2=\log_{10} e \log_{10}(1+\sigma_x^2/\xi^2). \tag{5}$$

Now $\sigma_x/\xi=V_x$ is the ratio of the standard deviation of x to its mean value and is the coefficient of variation of the untransformed data. Rewriting (5) we have

$$\sigma_y^2=0{\cdot}43428 \log_{10}(1+V_x^2),$$

from which it is seen that if the coefficient of variation of x is constant the variance of the transformed variate y will be constant and the methods of the 'analysis of variance' may be used. From equation (4) it is seen that the relation between the means of the transformed and untransformed distributions is not simple. For a sample the mean of the transformed values equals the logarithm of the *geometric mean* of the actual numbers.

Since $$y=\log_{10} x,$$

and $$\bar{y}=\frac{1}{n}\sum_{i=1}^{n} y_i=\frac{1}{n}\sum_{i=1}^{n}\log_{10} x_i=\log_{10}\sqrt[n]{(x_1\times x_2\times\ \dots\ \times x_n)},$$

i.e. $$\text{mean } y=\log_{10}\ (\text{geometric mean } x).$$

The mean of a sample drawn from a normal population is itself distributed normally with a variance equal to that of the parent population divided by the number in the sample, and therefore appropriate confidence limits may be calculated; since the *geometric mean* of a sample of n observations is related to the arithmetic mean of the transformed values in the same way as a single observation of x is to the transformed value $y=\log_{10} x$, for all values of n. If the limits for use with the arithmetic mean of the untransformed data are required, estimates of the population mean will have to be made from the actual numbers observed. Finney (1941) has shown that for data of this type the usual estimate of the sample mean, i.e. $\frac{1}{n}\sum_{i=1}^{n} x_i$ decreases in efficiency as an estimator of the population mean as the coefficient of variation increases. He has given corrections which can be used, but it is probably better to perform all comparisons on the transformed values, and thus also to avoid confusion between the geometric and arithmetic means.

THE METHOD OF WINSOR & CLARKE

The data considered by Winsor & Clarke consisted of catches from plankton nets hauled vertically, obliquely and horizontally. In each case similar methods of analysis were used. The observations were given in the form of estimated total catches of each of given groups of animals (defined by species, age and sex) calculated from laboratory samples, which represented numbers caught by nets hauled consecutively, and therefore effectively through the same body of water. The subsequent analysis of variance divided the total variance observed into its component parts.

In order that such an analysis of variance may be performed the data should satisfy several conditions, one of which is that the variances within different groups and due to different factors must be estimated on comparable scales, or 'equalized', and the resultant residual variations should be normal with zero mean. A transformation from the raw data may result in variates which satisfy these conditions.

Winsor & Clarke applied the logarithmic transformation and subsequent analysis of variance, which they used to estimate the variances and appropriate coefficients of variation. To illustrate this latter step it is best to quote from their paper.

> From the estimates 0·00781 and 0·00600 for the within haul and haul to haul variances, we obtain 0·0884 and 0·0775 as the estimated standard deviations. These are obtained from the logarithms of the catches. To interpret these figures in terms of variability of the actual catches we proceed as follows:
>
> $$0{\cdot}0884=\log 1{\cdot}226,$$
>
> $$0{\cdot}0775=\log 1{\cdot}195.$$
>
> Now a deviation of 0·0884 in the logarithm of the catch means that the catch has been multiplied (or divided) by 1·226. Hence we may say that one standard deviation in the logarithm corresponds to a percentage standard deviation, a coefficient of variation, of 22·6% in the catch. Similarly, a logarithmic standard deviation of 0·0775 corresponds to a coefficient of variation of 19·5%.

This implies that the relation between σ_y and V_x is

$$\sigma_y=\log_{10}(1+V_x), \tag{6}$$

but from equation (5) we see that this is not so. Equation (6) will always overestimate the value of V for a given σ_y, since it gives

$$V_x=10^{\sigma_y}-1, \tag{7}$$

whereas from (5)

$$V_x=\sqrt{(10^{\sigma_y^2/0{\cdot}43429}-1)}. \tag{8}$$

and the magnitude of the bias may be calculated. For the case quoted by Winsor & Clarke, calculation from equation (5) gives

$$\sigma_x^2=0{\cdot}00781=0{\cdot}43429\,(1+V_x^2),$$

$$1+V_x^2=\text{antilog}_{10}\frac{(0{\cdot}00781)}{(0{\cdot}43429)}=1{\cdot}0422,$$

and

$$V_x=0{\cdot}205 \quad \text{or} \quad 20{\cdot}5\%$$

for which Winsor & Clarke obtained 22·6%.

For $\sigma_y^2=0{\cdot}0400$ eqn. (5) gives 48·6% and the method of Winsor & Clarke 58·5%; and $\sigma_y^2=0{\cdot}0900$ eqn. (5) gives 78·2% and the method of Winsor & Clarke 99·5%.

The bias due to the method of Winsor & Clarke increases with increase in σ_y^2.

THE METHOD OF SILLIMAN

Silliman's data consisted of observations on the number of pilchard eggs in hauls made with two identical nets, each hauled once at a number of different stations. The data was not given as estimated catch, but as the numbers counted in laboratory samples. Two laboratory samples were taken from each haul and both counts were given. This data, like that of Winsor & Clarke, has a constant coefficient of variation, and a logarithmic transformation was used. The analysis of variance showed a highly significant variation between stations; estimates of the 'within station between haul' and the 'between samples' variances were obtained.

Winsor & Clarke suggested the use of confidence limits in order to compare catches, and if these are calculated from the numbers obtained in several hauls from a given station, or at a given time, they provide a test of significance for a further observation from, say, a different station, or at a different time.

From equations (4) and (5) it is seen that the 95 % limits for the untransformed distribution correspond to the antilogarithms of the values for the 95 % limits for the transformed (normal) distribution; and are therefore $\text{antilog}_{10}\,(\eta - t\sigma_y)$ and $\text{antilog}_{10}\,(\eta + t\sigma_y)$. If these are to be expressed as proportions of the mean of the untransformed distributions, we have

$$\frac{\text{antilog}_{10}\,(\eta - t\sigma_y)}{\xi} \quad \text{and} \quad \frac{\text{antilog}_{10}\,(\eta + t\sigma_y)}{\xi}$$

$$\text{or} \quad \log_{10}\frac{(\text{lower limit})}{\text{mean}} = -t\sqrt{[0{\cdot}43429 \log_{10}(1+V_x^2)]} - \tfrac{1}{2}\log_{10}(1+V_x^2), \qquad (9)$$

$$\log_{10}\frac{(\text{upper limit})}{\text{mean}} = +t\sqrt{[0{\cdot}43429 \log_{10}(1+V_x^2)]} - \tfrac{1}{2}\log_{10}(1+V_x^2), \qquad (10)$$

in which form they are expressed in terms of the coefficient of variation only. Silliman, recognizing this property, has argued as follows:

> Solution of these equations gives $\sigma_H^2 = 0{\cdot}024$ as the variance of hauls and $\sigma_S^2 = 0{\cdot}007$ as the variance of samples. Therefore $\sigma_H = \sqrt{0{\cdot}024} = 0{\cdot}155$ and $\sigma_S = \sqrt{0{\cdot}007} = 0{\cdot}084$. The 95 % fiducial limits are of interest and may be calculated from these estimates of σ. For hauls $2 \times 0{\cdot}155 = 0{\cdot}310$ (2σ limits include 95 % of the distribution). Since these values are logarithmic, the antilogs are used to convert to ratios. The antilog of 0·310 is 2·04 giving fiducial limits of 49 % ($100 \times 1/2{\cdot}04$) to 204 % ($100 \times 2{\cdot}04$). Thus the egg number from one haul may not be considered significantly different from the egg number at another, unless it is less than one half, or more than double, that of the other. Similarly, the 95 % fiducial limits for samples are 68 % to 147 %, and may be interpreted in a like manner.

This method implies that the ratios are

$$\frac{\text{antilog}_{10}\,(\eta - 2\sigma_y)}{\text{antilog}_{10}\,\eta} \quad \text{and} \quad \frac{\text{antilog}_{10}\,(\eta + 2\sigma_y)}{\text{antilog}_{10}\,\eta},$$

i.e. the mean employed is the antilogarithm of the mean of the transformed distribution, the geometric mean of the untransformed data. Similarly, estimates of the confidence limits for the mean of a sample are expressed as percentages of the geometric mean of the sample. Throughout Silliman has used the word mean, which is generally taken to apply only to the arithmetic mean.

If the arithmetic mean is used in this way, where the method is strictly only applicable to the geometric mean, anomalous results may be obtained. A numerical example taken from Silliman's data may illustrate the different results obtained with the use of the geometric and arithmetic means.

The laboratory counts of pilchard eggs for two samples drawn from each of two hauls taken at twenty-four different stations were given. Do the mean values of the first samples differ significantly between the two hauls? The means of the first samples are:

	Haul A	Haul B
Mean of actual numbers	70·5	85·6
Mean of logarithmic values	1·6499	1·7031
Geometric mean catch	44·66	50·48

The variance to be associated with a single haul, obtained from the analysis of variance, and based on 69 degrees of freedom, is 0·031.

The variance of the mean of 24 hauls is therefore 0·031/24
and the standard deviation 0·0359
2 × standard deviations* 0·0718

and the confidence limits are antilog $1{\cdot}6499 + 0{\cdot}0718 = 52{\cdot}69$
antilog $1{\cdot}6499 - 0{\cdot}0718 = 37{\cdot}85$.

From which it is seen that the geometric mean of the samples for the B hauls falls within the confidence limits derived from the A hauls. If the limits are calculated following Silliman and applied to the arithmetic mean of the untransformed observations, they are:

Lower limit 84·7% of $70{\cdot}5 = 59{\cdot}11$,
Upper limit 117% of $70{\cdot}5 = 83{\cdot}19$,

and the observed value of 85·6 for the B hauls appears to be significant as it falls outside the confidence limits derived from the A hauls. This result differs from that obtained above.

For confidence limits for a single observation the limits in actual numbers given by the two methods are equal as there is no difference between the estimate of the arithmetic mean and the geometric mean for a sample of one.

DISCUSSION

Although the estimates of the coefficient of variation as given by Winsor & Clarke have been shown to have been incorrectly obtained, the correct coefficient of variation does give a useful indication of the variability to be expected with any particular method and gear. The estimates given in this paper are lower than those given by them, but for their data are nevertheless high. For critical comparisons of catches, using the same gear and method confidence limits are more useful. These may be calculated from the transformed data and expressed as actual numbers for a single observation, or as limits for the geometric mean of a number of catches calculated as percentages of the geometric mean of the observed data. This is equivalent to performing the comparisons on the logarithmic values.

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* In this case $t = 2$ for 95% probability level and 69 degrees of freedom.

It must be emphasized that the derivation of estimates, both of the coefficient of variation and of confidence limits, given in this paper, relies on the assumption that the logarithmic transformation employed has normalized the observed distribution. In practice this will not be completely realized, but the estimates given will be better than those obtained by the methods of Winsor & Clarke and Silliman, which take little account of the mathematical relations involved.

For some observed data, a logarithmic transformation of the form $y = \log_{10}(x+a)$ is more appropriate and, from the equations given above, estimates of the coefficient of variation can be obtained. For such a transformation, the variance of the untransformed data remains σ_x^2, but the mean becomes $(\xi + a)$. From Fig. 1 it will be seen that confidence limits for a single observation depend only on area, and may therefore be obtained for any *normalizing* transformation, even when the mathematical relations between the parameters of the two distributions are not readily available. Comparisons of sample means may be carried out on the transformed values.

Thanks are due to Mr N. W. Please for his helpful comments on this paper.

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THE GROWTH RATE OF THE LONG ROUGH DAB *HIPPOGLOSSOIDES PLATESSOIDES* (FABR.)

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(Plate I and Text-figs. 1–6)

The Long Rough Dab, *Hippoglossoides platessoides* (Fabr.), is a common fish in the Clyde Sea Area, being indeed the most abundant flatfish. However, despite its abundance and its wide geographic range (it is found from the British coasts as far north as Spitzbergen and the Murman coast, Iceland, Greenland and south to Cape Cod in America), very little work has been done on its general biology. This is no doubt owing to its having little or no economic value. Concerning its growth rate, which is the subject of this paper, the European subspecies *limandoides* (Bloch) (see Norman, 1934) has been investigated to some extent in the Barents Sea (Essipow & Slastnikow, 1932; and Milinsky, 1944), and has been mentioned in more general papers by Saemundsson (1925) and Krüger (1942) for Icelandic and Baltic populations respectively. The American population which constitutes a separate subspecies *platessoides*, has been discussed by Huntsman (1918), though he does not give much numerical data on the growth rate.

In the work described below the ages of 1561 *H. platessoides limandoides* caught in the Clyde area have been determined and their growth rate calculated.

GEAR

The gear used in obtaining the samples on which this work is based was a small-mesh cotton V.D. trawl of the following dimensions: headline, 49 ft.; foot rope, 78 ft.; bridles, 15 fathoms; lower wings and belly mesh size, $2\frac{1}{2}$ in. bar; all other meshes, 1 in. bar. This gear took an adequate sample of the population, except that the mesh-size selection gave a noticeably biased sample of the younger age-groups. This point will be discussed in a later section (p. 305). In order to illustrate the adequacy of the gear in obtaining representative samples two tests were carried out on a pair of hauls taken over the same ground on 8 July 1954. In the first test (see Table 1) the mean lengths of the age-groups present were compared and no significant differences were found. In the second test the numbers of fish of each age-group were compared, and again no significant differences were found.

THE MATERIAL AND METHODS

The samples were obtained monthly, and for the most part the fish were caught in an area at about 40 m. depth off Mountstuart House on the east side of the Isle of Bute. Before this area was finally chosen some of the earlier hauls were taken on various grounds round the Isle of Cumbrae, and prior to the spawning time hauls were also taken in the deeper water between Bute and Cumbrae. Owing to the depth segregation of spawning and immature fish, and so also of the relative proportions of different sizes and ages, these latter hauls led to the samples being more representative of the population as a whole.

TABLE I. COMPARISON OF TWO REPLICATE CATCHES OF LONG ROUGH DABS

	Haul I		Haul II	
Age-group	Number	Mean length (cm)	Number	Mean length (cm)
2	15	13·2	12	13·4
3	33	18·8	26	18·3
4	22	23·1	24	22·7
5	9	23·1	5	24·0

The dates on which the samples were obtained are given in Table II.

The examination of the fish after capture was carried out in the laboratory, and for the purposes of this investigation, the sex and length of each fish was noted and the otoliths were extracted and stored in separate packets. The lengths were measured from the tip of the lower jaw to the end of the longest caudal fin ray to the nearest 0·5 cm. These measurements however were later grouped into centimetre groups, and the resulting groups of from $x-0{\cdot}25$ cm to $x+0{\cdot}75$ cm were all classed as x cm rather than $x.25$ cm for ease in computation.

AGE DETERMINATION

The ages of the fish were determined from the otoliths (Pl. I). These were examined as soon as possible after extraction, in a strongly illuminated black dish. They were found to be very clear and easy to read. The number of translucent rings that alternate with opaque bands was noted and taken to represent the age of the fish in years.

In order to test the accuracy of the otolith readings some scales were examined from a few fish, but they proved to be very obscure and difficult to read and the attempt was abandoned. However, the edges of the otoliths were examined and the percentage that were translucent was calculated. The data are given in Table III and it can be seen that the assumption that the rings are annual structures is confirmed.

From the age analysis each fish was allocated to a population *a–f* dependent on when it was supposed to have been spawned. This obviates the difficulty during the winter and spring when fish that were spawned in a given year may have a different number of translucent rings dependent on the condition of the outer edge.

TABLE II. DETAILS OF LONG ROUGH DAB CATCHES

lation ...	*a*		*b*		*c*		*d*		*e*		*f*		Totals		Grand totals
...	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	
Oct.	—	—	—	4	1	13	—	22	1	21	5	5	7	65	72
Nov.	—	—	—	—	3	9	2	15	3	18	2	9	10	51	61
Dec.	—	—	—	2	1	5	1	17	1	18	2	6	5	48	53
Jan. Jan.	—	—	—	6	1	21	1	28	2	22	1	3	5	80	85
Feb.	—	—	—	3	—	12	—	30	—	4	—	—	—	49	49
Mar.	—	—	—	7	2	26	2	78	4	149	1	8	9	268	277
Mar.	—	4	—	3	—	18	—	36	—	44	—	—	—	105	105
Apr.	—	1	—	18	—	27	—	52	—	48	—	1	—	147	147
May	—	—	—	8	1	15	2	30	2	59	—	5	5	117	122
June	—	1	—	2	4	16	12	54	10	98	3	6	29	177	206
July	—	—	—	1	1	14	7	46	5	59	3	15	16	135	151
Aug.	—	—	—	1	4	10	13	36	15	46	5	23	37	116	153
Sept.	—	—	—	2	1	5	8	16	—	35	3	10	12	68	80
Total	—	6	—	57	19	191	48	460	43	621	25	91	135	1426	1561

TABLE III. PERCENTAGE OF OTOLITHS WITH TRANSLUCENT EDGES

Oct.	36·5	Apr.	50·2
Nov.	46·1	May	10·7
Dec.	75·2	June	18·0
Jan.	74·2	July	7·4
Feb.	83·7	Aug.	10·6
Mar.	74·3	Sept.	15·2

GROWTH RATE

In the analysis of the growth rate the two sexes were treated separately. For each population sample in each month the mean length was calculated, and its 95% fiducial limits obtained for all samples where sufficient numbers were caught. The data for the females are given in Table IV and for the males in Table V, though for the latter the numbers are unfortunately too small to make calculation of fiducial limits worth while. It can immediately be seen that the males are not only of a smaller size than females of a corresponding age, but also that they have a shorter life span (see also Fig. 3).

The data in Table IV are shown in Fig. 1. From this it can be seen that the most rapid growth takes place in the younger fish and falls off in later years. The seasonal differences in growth rate can also be seen, the faster growth taking place between April and November. It will be noticed that this period of faster growth is at a time when the edge of the majority of otoliths is opaque

TABLE IV. MEAN LENGTHS (AND THEIR FIDUCIAL LIMITS) OF FEMALE LONG ROUGH DABS FROM POPULATIONS *a* TO *f*

Population ...	*a*	*b*			*c*			*d*			*e*			*f*		
	Mean	Lower limit	Mean	Upper limit	Lower limit	Mean	Upper limit	Lower limit	Mean	Upper limit	Lower limit	Mean	Upper limit	Lower limit	Mean	Upper limit
Oct.	—	—	25·5	—	22·0	23·4	24·7	21·2	21·9	22·7	16·3	17·0	17·8	—	10·0	—
Nov.	—	—	—	—	20·8	22·7	24·4	20·4	21·6	22·8	16·6	17·6	18·5	11·1	12·3	13·6
Dec.	—	—	17·5	—	17·7	21·0	24·3	19·8	21·1	22·3	17·1	18·0	18·9	11·0	12·7	14·3
Jan.	—	24·2	26·3	28·5	23·1	24·1	25·2	21·1	21·7	22·3	16·9	17·8	18·7	—	11·7	—
Feb.	—	—	27·7	—	23·1	24·5	25·9	22·3	22·8	23·3	17·2	18·5	19·9	—	—	—
Mar.	—	24·7	26·4	28·2	21·7	22·7	23·6	21·3	21·5	21·7	17·1	17·4	17·7	—	9·8	—
Mar.	26	—	25·3	—	21·5	22·6	23·8	21·3	22·0	22·6	17·9	18·5	19·1	—	—	—
Apr.	22	23·4	24·4	25·5	22·1	23·1	23·9	21·7	22·1	22·6	18·0	18·6	19·1	—	11	—
May	—	23·1	25·3	27·4	22·1	23·7	25·3	21·3	22·2	23·1	17·6	18·2	18·7	—	10·8	—
June	26	—	25·0	—	23·2	24·5	25·8	21·8	22·5	23·1	17·8	18·2	18·6	10·7	12·0	13·3
July	—	—	21	—	22·5	23·4	24·4	22·3	22·9	23·5	18·0	18·5	19·0	12·4	13·2	14·0
Aug.	—	—	23	—	22·1	24·1	26·1	21·5	22·1	22·8	19·4	19·9	20·4	13·0	13·6	14·2
Sept.	—	—	24·5	—	21·7	23·8	25·9	21·5	22·8	24·0	19·5	20·3	21·0	13·4	14·6	15·8
Mean for the year	25·3	24·7	25·3	26·0	23·1	23·4	23·7	21·9	22·1	22·3	18·1	18·3	18·4	12·2	12·6	13·0

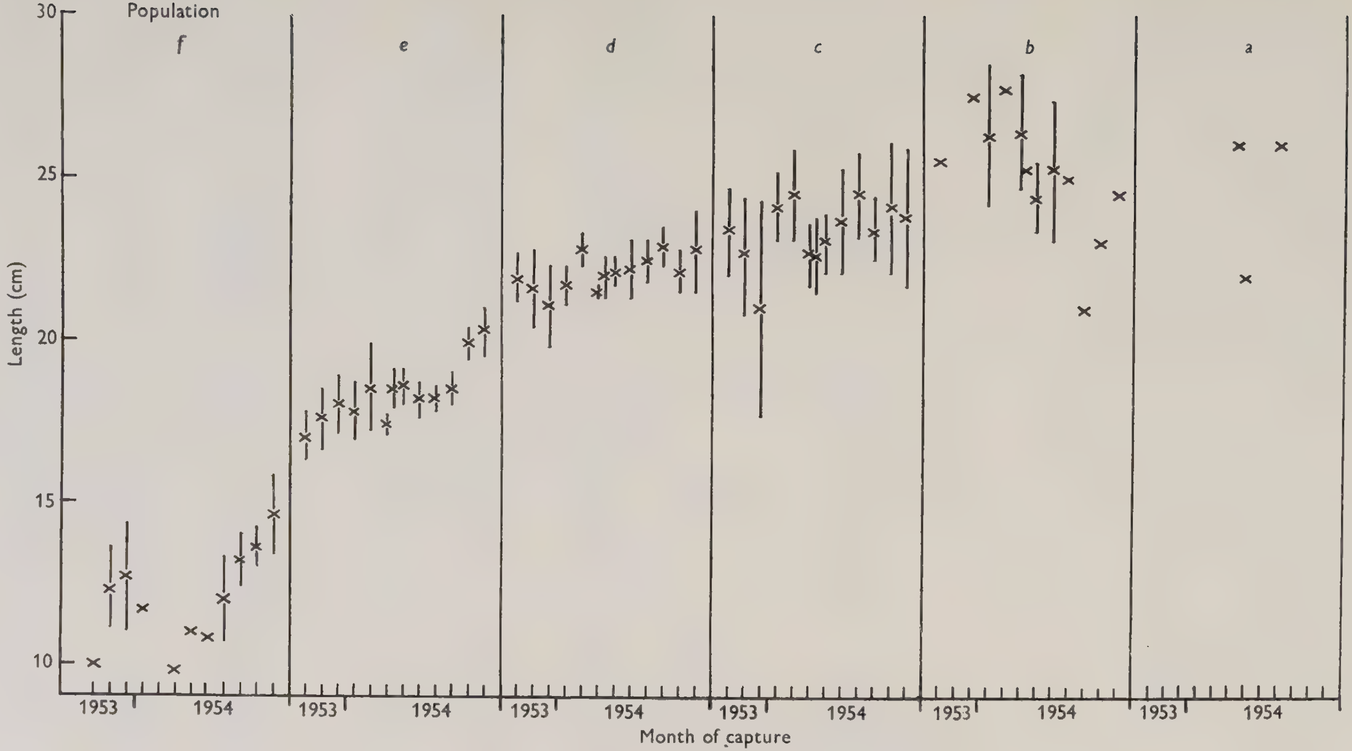


Fig. 1. The means (×) and their 95% fiducial limits (vertical lines) of female Long Rough Dabs of six populations, *a–f*, from samples taken from October 1953 to September 1954

(Table III). In Fig. 1 it must be remembered that it is not samples from one generation over 6 years that are shown, but of six age-groups sampled over 1 year. The means for the year that are given in Tables IV and V can reasonably be taken as the mean lengths after 1 to 6 years (for populations *f* to *a*), since it can be shown that the Long Rough Dab spawns at the beginning of April, that is, at about the mid-point in the period from October to the following September when the samples described in the tables were taken.

TABLE V. MEAN LENGTHS OF MALE LONG ROUGH DABS FROM POPULATIONS *c* TO *f*

Population ...	*c*	*d*	*e*	*f*
Oct.	14	—	11	8·8
Nov.	17·3	13·5	12·0	10·5
Dec.	14	15	14	10·5
Jan.	15	17	13·0	10
Feb.	—	—	—	—
Mar.	16·0	15·0	13·0	13
Apr.	—	—	—	—
May	19	16·0	13·5	—
June	16·0	14·4	13·0	11·0
July	14	15·9	13·8	11·3
Aug.	17·0	15·9	14·3	10·8
Sept.	17	15·5	—	10·7
Mean for the year	16·3	15·3	13·5	10·5
Lower limit	15·6	14·9	13·0	9·9
Upper limit	17·0	15·7	14·0	11·0

The mathematical treatment of these data can be based on the regression of $l_{(n+1)}$ on l_n (where l_n equals the mean length at each age n). The regression equation, which is the same as that used by Ford (1933) and Walford (1946), is

$$l_{(n+1)} = ml_n + C. \tag{i}$$

The slope of the line, m, is that proportion of the previous length added to the constant C, which is the mean length of the fish after 1 year. Since m always has a value of less than 1, the growth is of an exponential form, tending to an asymptotic value (l_∞) and this can be derived from equation (i) as

$$l_\infty = \frac{C}{(1-m)}. \tag{ii}$$

A suitable exponential formula, established on general physiological principles by Bertalanffy (1938, 1949) and as used by Parrish & Jones (1953), is

$$l_t = l_\infty(1-e^{-kt}), \tag{iii}$$

where l_t is the length at any time t, and k is a constant. This will be equivalent to equation (i) if

$$\left.\begin{aligned} l_\infty &= \frac{C}{(1-m)}, \\ e^{-k} &= m, \end{aligned}\right\} \tag{iv}$$

and the constant (C) is the length at 1 year old. This last condition is necessary because the exponential equation passes through the origin, whereas the regression allows a value for l_0 (which may perhaps represent the length of the fish when the otolith formation commences). However, if a comparison of the regression equation and the data suggests that a value for l_0 should be calculated, this can be obtained from equation (i), and if one wishes to fit the exponential equation (iii) so that l_0 has this value, the origin of t must be changed by an amount x, such that the lengths are calculated for values of $t=n+x$. Equation (iii) now becomes:

$$l_n = l_\infty(1-m^{n+x}), \tag{v}$$

and writing $n=0$ we get

$$x=\frac{\log(l_\infty - l_0)-\log l_\infty}{\log m}. \tag{vi}$$

In the analysis of the data by these methods, the regression equations (i) were calculated from the mean lengths given in Tables IV and V, care being taken to weight the points in proportion to the number of fish contributing to each mean. The equations found were

$$\left.\begin{array}{ll}\text{for the females: } & l_{n+1}=0\cdot574\, l_n+11\cdot262,\\ \text{for the males: } & l_{n+1}=0\cdot585\, l_n+7\cdot368;\end{array}\right\} \tag{vii}$$

and the l_∞ values therefore were given by

$$\left.\begin{array}{ll}\text{for the females: } & l_\infty=\dfrac{11\cdot262}{(1-0\cdot574)}=26\cdot437 \text{ cm},\\[2ex] \text{for the males: } & l_\infty=\dfrac{7\cdot368}{(1-0\cdot585)}=17\cdot754 \text{ cm}.\end{array}\right\} \tag{viii}$$

These values in the exponential equation (3) give

$$\left.\begin{array}{ll}\text{for the females: } & l_t=26\cdot437\,(1-0\cdot574^t),\\ \text{for the males: } & l_t=17\cdot754\,(1-0\cdot585^t).\end{array}\right\} \tag{ix}$$

Successive values of l_t are given in Table VI and Fig. 2. It will be noticed that the constant (C) in equation (vii) is not equal to the observed l_1 so that a value for l_0 can be obtained. If this is calculated from l_1

$$\left.\begin{array}{ll}\text{for the females: } & l_0=2\cdot33 \text{ cm},\\ \text{for the males: } & l_0=5\cdot35 \text{ cm}.\end{array}\right\} \tag{x}$$

The accuracy of these values is dependent on the accuracy of l_1, and a better estimate can be obtained from the data as a whole by finding a weighted l_0 from all the age groups (using equation (vii)). This method gives

$$\left.\begin{aligned}\text{for the females: } & l_0=2{\cdot}130 \text{ cm,}\\ \text{for the males: } & l_0=5{\cdot}335 \text{ cm.}\end{aligned}\right\} \qquad \text{(xi)}$$

Using these values in equation (vi) we get

$$\left.\begin{aligned}\text{for the females: } & x=0{\cdot}151 \text{ years,}\\ \text{for the males: } & x=0{\cdot}667 \text{ years;}\end{aligned}\right\} \qquad \text{(xii)}$$

TABLE VI. SUCCESSIVE VALUES OF l_t, FROM EQUATION (ix)

	Females		Males	
t	Observed	Calculated	Observed	Calculated
1	12·6	11·3	10·5	7·4
2	18·3	17·7	13·5	11·7
3	22·1	21·4	15·3	14·2
4	23·4	23·6	16·3	15·7
5	25·3	24·8	—	—
6	25·3	25·5	—	—

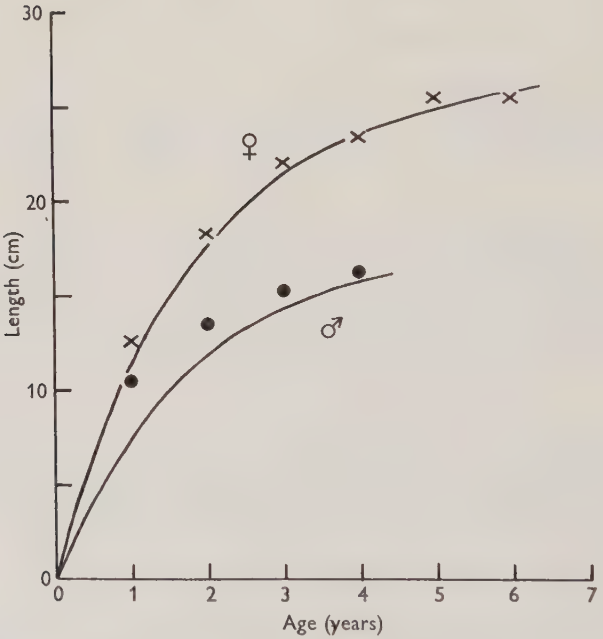


Fig. 2. Observed means and generalized age/length relation of Long Rough Dabs. Females (×), with curve $l_t=26{\cdot}437\ (1-0{\cdot}574^t)$. Males (●), with curve $l_t=17{\cdot}754\ (1-0{\cdot}585^t)$.

and so equation (v) becomes

$$\left.\begin{aligned}\text{for the females: }\; l_n&=26{\cdot}437\,(1-0{\cdot}574^{n+0{\cdot}151}),\\ \text{for the males: }\; l_n&=17{\cdot}754\,(1-0{\cdot}585^{n+0{\cdot}667}).\end{aligned}\right\}\qquad\text{(xiii)}$$

Successive values of l_n are given in Table VII.

TABLE VII. SUCCESSIVE VALUES OF l_n, FROM EQUATION (xiii)

Males				Females			
Observed		Calculated		Observed		Calculated	
$n=1$	10·5	$n=1{\cdot}667$	10·49	$n=1$	12·6	$n=1{\cdot}151$	12·48
2	13·5	2·667	13·51	2	18·3	2·151	18·44
3	15·3	3·667	15·27	3	22·1	3·151	21·84
4	16·3	4·667	16·30	4	23·4	4·151	23·80
	—		—	5	25·3	5·151	24·92
	—		—	6	25·3	6·151	25·57

If the lengths given by equation (xiii), which fit the data very well, are the best mathematical approximations to the population, we must examine the meaning of the change x in the time scale; and various hypotheses can be suggested.

First, the values of l_0 may represent the length of the fish when the otolith formation commenced—or the otolith formation does not start until the fish are x years old—but if this were true it is difficult to see why the values are so different for the males and females. A second interpretation would be to suppose that the value of x may represent the difference between the true anniversary of spawning and that implied in the observed data. This can only mean that the males and females are produced at quite different times, which is absurd.

A third interpretation supposes the sampling contains an inherent bias. It has so far been assumed that the observed mean lengths ($\bar{x}$) are the best estimates of the population means (ξ). If, however, we allow that the sample means may be biased and do not necessarily approximate to the population values, it is suggested that the apparently good fit given in Table VII is a spurious one. The type of factor which would lead up to the kind of bias we are considering might be net mesh-size selection. With such a factor the observed mean lengths would all be too large (cf. Table VI), but if the bias for l_n and $l_{(n+1)}$ were even approximately the same, the biased mean lengths of the sample would give similar values of m and C in equation (i) as would the correct population means, since the regression is based on the ratio of l_{n+1} to l_n and not on their absolute values. This can be seen from the equation,

$$m=\frac{l_{n+1}-l_n}{l_n-l_{n-1}},$$

since if the mean lengths are similarly biased, the amounts of bias will cancel each other. If this interpretation is correct the calculated mean lengths given in Table VI are more likely to represent those of the population than are the observed sample means, and the difference is produced by a factor such as net selection.

TABLE VIII

	Females						Males			
Age	1	2	3	4	5	6	1	2	3	4
$\bar{x}$	12·6	18·3	22·1	23·4	25·3	25·3	10·5	13·5	15·3	16·3
Approximate upper range limit	19	26	30	31	31	?	14·5	18	19	20
ξ	11·3	17·7	21·4	23·6	24·8	25·5	7·4	11·7	14·2	15·7
3σ	7·7	8·3	8·6	7·4	6·2	—	7·0	6·3	4·8	4·4
$\xi+2\sigma$	16·5	23·3	27·2	28·6	29·0	—	12·0	15·9	17·4	18·6
$\xi-2\sigma$	6·1	12·1	15·6	18·6	20·6	—	2·8	7·5	11·0	12·6

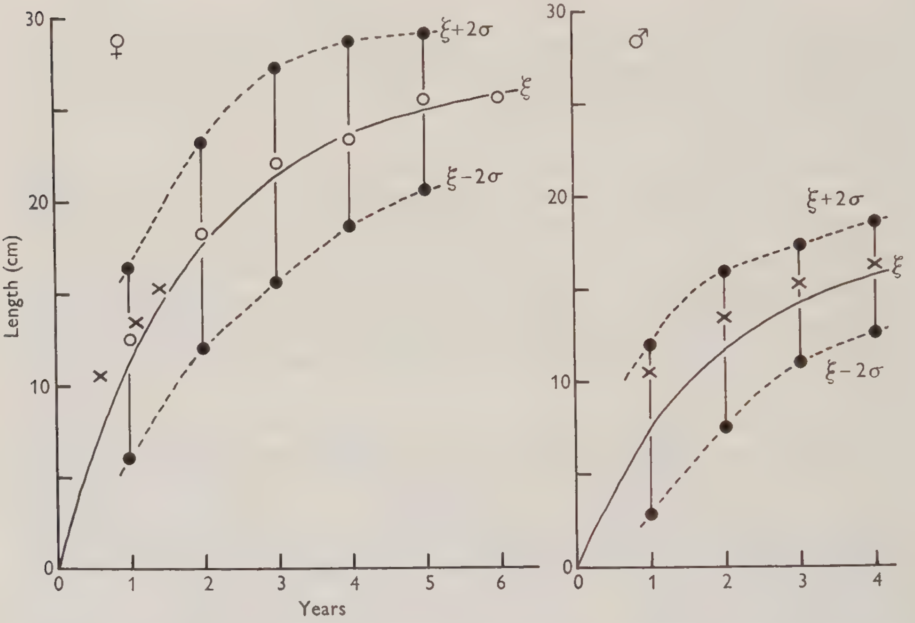


Fig. 3. Diagrams to illustrate the relation of theoretical population means (ξ) and the sample means ($\bar{x}$): ○·····○ for females; ×·····× for males. (In the left-hand diagram × represents $\bar{x}$ for males of comparable theoretical size, having been simply transferred from the right-hand diagram.)

Since net-selection bias operates mostly on the smaller fish, we may take the largest observed fish of any age-group to indicate the upper limit of the population range, and if we assume that the length frequency is normally distributed we may take this to represent three standard deviations and thus we may deduce the 2σ limits about the mean which should include 95% of the population. These values have been calculated for the males and females and are given in Table VIII and Fig. 3. It will be noticed that the observed lengths are within the 2σ limits. It can also be seen from Fig. 3 that the discrepancy between the males and females is very small; the observed mean lengths for the first three age-groups of the males have been transposed to the appropriate position on the graph for the females, and it can be seen that the net selection bias operates very similarly for comparable lengths of both sexes.

The hypothesis of net-selection bias being the reason for the discrepancy between the observed and calculated lengths for each age is not put forward to the exclusion of the other factors given above. It is very unlikely that the observed lengths are those appropriate for whole numbers of years after spawning or the time of otolith formation; nevertheless, it is believed that a hypothesis concerning net selection, or some similar factor, is necessary for a true understanding of the facts. If, because of the time of sampling, it is necessary to change the value of t, it must be remembered that the otolith formation will probably commence at the same length in the males and females, so that the lengths at l_0 should be the same, but that this will be reached sooner by the faster growing sex, which, in Long Rough Dabs of this size, are the females.

TABLE IX. CALCULATED VALUES OF l_t, FROM EQUATION (iii), WHERE $m=0{\cdot}4897$

Age	Observed	Calculated
1	10·5	9·1
2	13·5	13·5
3	15·3	15·7
4	16·3	16·7

The mathematical treatment of the data in this paper has been based on the regression line of $l_{(n+1)}$ on l_n, which is the 'best' straight line obtained by the method of least squares, weighted according to the number contributing to the mean length for each age-group. The values of the growth constants obtained in this way are more satisfactory and objective than any that could be obtained by fitting the curve of equation (iii) to the data by trial and error; or by purely graphical methods. The real advantage of the method used in this paper will be appreciated if we consider the growth of the males. By substituting the observed lengths into equation (iv) a weighted mean value for k can be obtained and has been found to be 0·714, corresponding to $m=0{\cdot}4897$. The calculated values of l_t using this figure in equation (iii) are given in Table IX and give what is apparently a good fit, but there are numerous objections to the method.

It is always easier and more accurate to fit a straight line than a curve; and since the line is based on ratios, if there is bias in the mean lengths which is even approximately similar, better results will be obtained than by using the biased observed values separately. Secondly, the slope of the line is not 0·4897, and it can hardly be fortuitous that the values of m in equation (i) are so similar for both sexes. Furthermore, Bertalanffy (1938) showed that a curve of the form in equation (iii) could be established on physiological principles. If his reasoning is correct the curve should fit the observed data, and any discrepancy will most probably be due to some factor such as biased

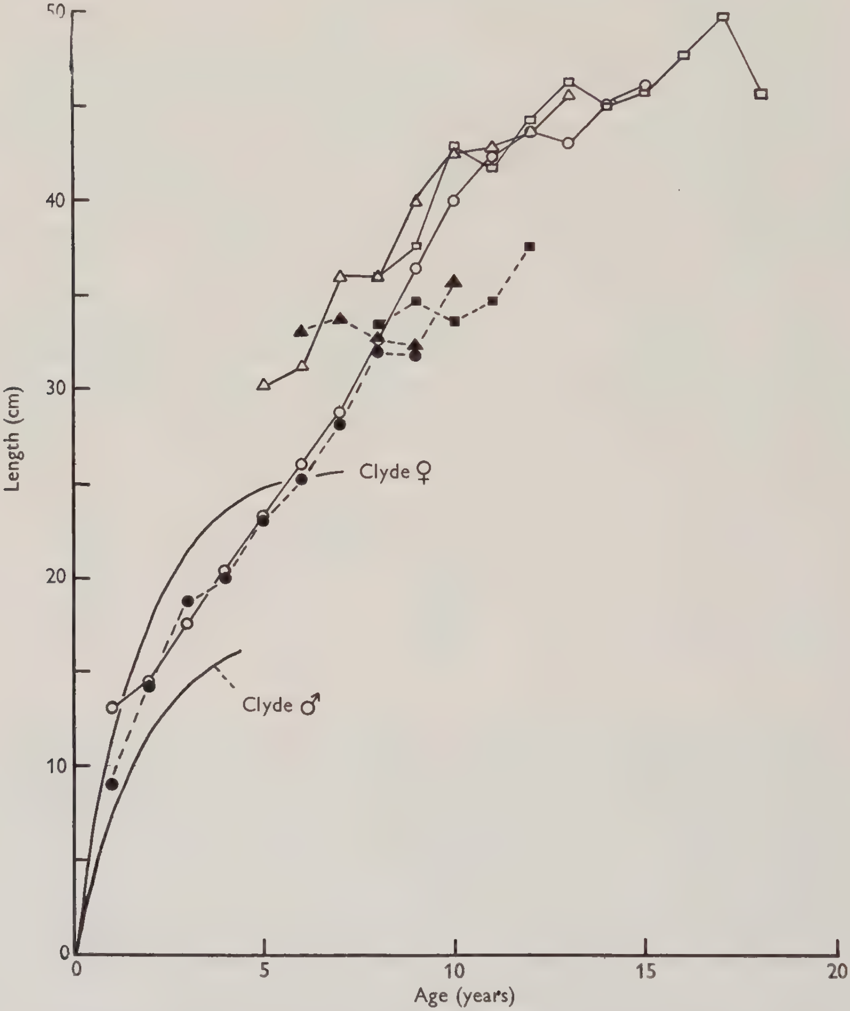


Fig. 4. Comparison of Clyde Long Rough Dab growth rate with that given by Milinsky (○ females, ● males, from Table II; □ females, ■ males from Table III) and by Essipow & Slastnikow (△, females, ▲ males) for the Barents Sea.

sampling or faulty age determination. The discrepancies found in Table VI can be explained very satisfactorily in this way, but it is hard to see why in Table IX the observed values for both $t=3$ and $t=4$ are too low. It is very important in work of this kind to bear in mind the limitations of the sampling device and to anticipate what differences these may produce on the parameters of the sample as compared with those of the population.

Comparison with Previous Studies

The most striking point that emerges from a comparison of the age and growth of Clyde-caught Long Rough Dabs and those from elsewhere is the difference in longevity. The ages recorded for Barents Sea specimens given by Essipow & Slastnikow (1932) range from 5 to 13, and Milinsky (1944) records females of up to 19 years old. Similarly, in Icelandic populations, Saemundsson (1925) found Long Rough Dabs of 17 years old, and Huntsman (1918), discussing the American subspecies *H. p. platessoides*, states that they can certainly live as long as 24 years, and probably 30 years should be assigned as the upper limit. In the Clyde no specimens have been found that have survived their sixth year.

Similar differences are found in the maximum sizes to which the fish grow. Specimens of 48·0 cm (Essipow & Slastnikow, 1932) and of over 50 cm (Milinsky, 1944) have been recorded from the Barents Sea, and of 2 ft. (61 cm) from Canada (Huntsman, 1918). Saemundsson (1925) found Long Rough Dabs of up to 42 cm at Iceland, and the longest recorded by Krüger (1942) for the Baltic was 34 cm. The largest specimen in the 1561 Clyde-caught Long Rough Dabs was 30·5 cm long.

With the rate of growth, however, no such very great differences are found, particularly in the younger age-groups. The data extracted from the Russian papers are shown in Fig. 4, together with the curves for the Clyde populations. It can be seen that the initial rates of growth are quite comparable, though later differing greatly, and the sexual differences are not apparent in the Russian data. Huntsman does not give any numerical data except as a generalized graph to illustrate that the growth rate varies markedly from place to place, and this is attributed to water-temperature differences. Saemundsson, however, gives sufficient data from Iceland for a comparison to be made between the Clyde and Icelandic populations. This is shown graphically in Fig. 6, which illustrates the data for 11–25 June 1924, chosen because it is sufficiently large and quite representative of the other data given.

Krüger's growth rate data from the Baltic (the nearest recorded to the Clyde) is shown in Fig. 6, where it can be seen that the rates are very similar, except for the older females, though the males are almost identical.

SUMMARY

The ages of 1426 female, and 135 male Long Rough Dabs have been determined from otolith readings. The females were found to live into their seventh year and reach 30·5 cm, compared with males that live to their fifth year and reach 19·0 cm in length. However, the mean increase in length per year for a given length, has been found to be the same. The growth of the females has been shown to fit the equation $l_t = 26{\cdot}437\ (1 - 0{\cdot}574^t)$ and of the males

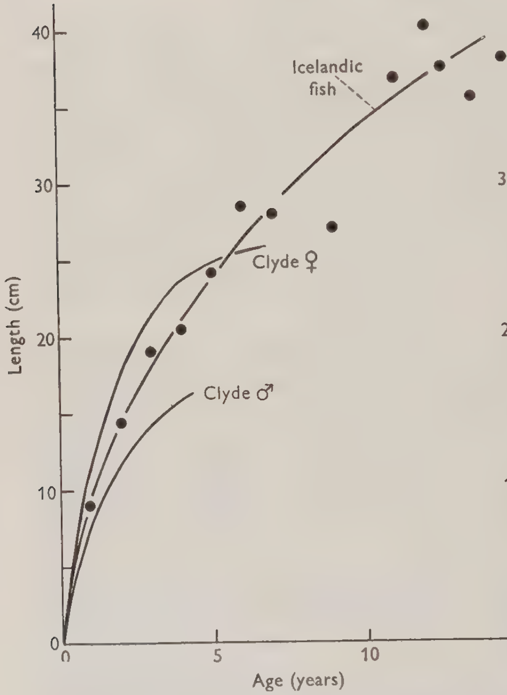


Fig. 5. Growth rate comparison of Clyde and Icelandic Long Rough Dabs.

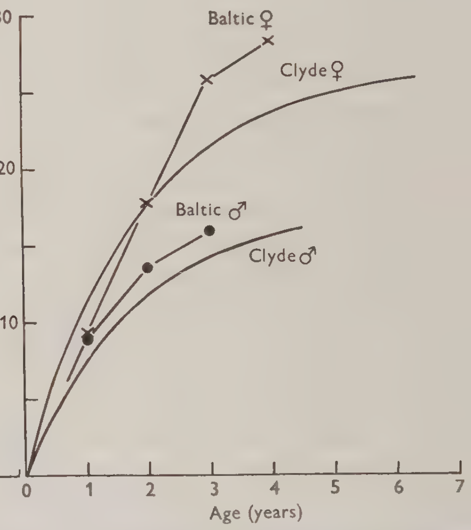
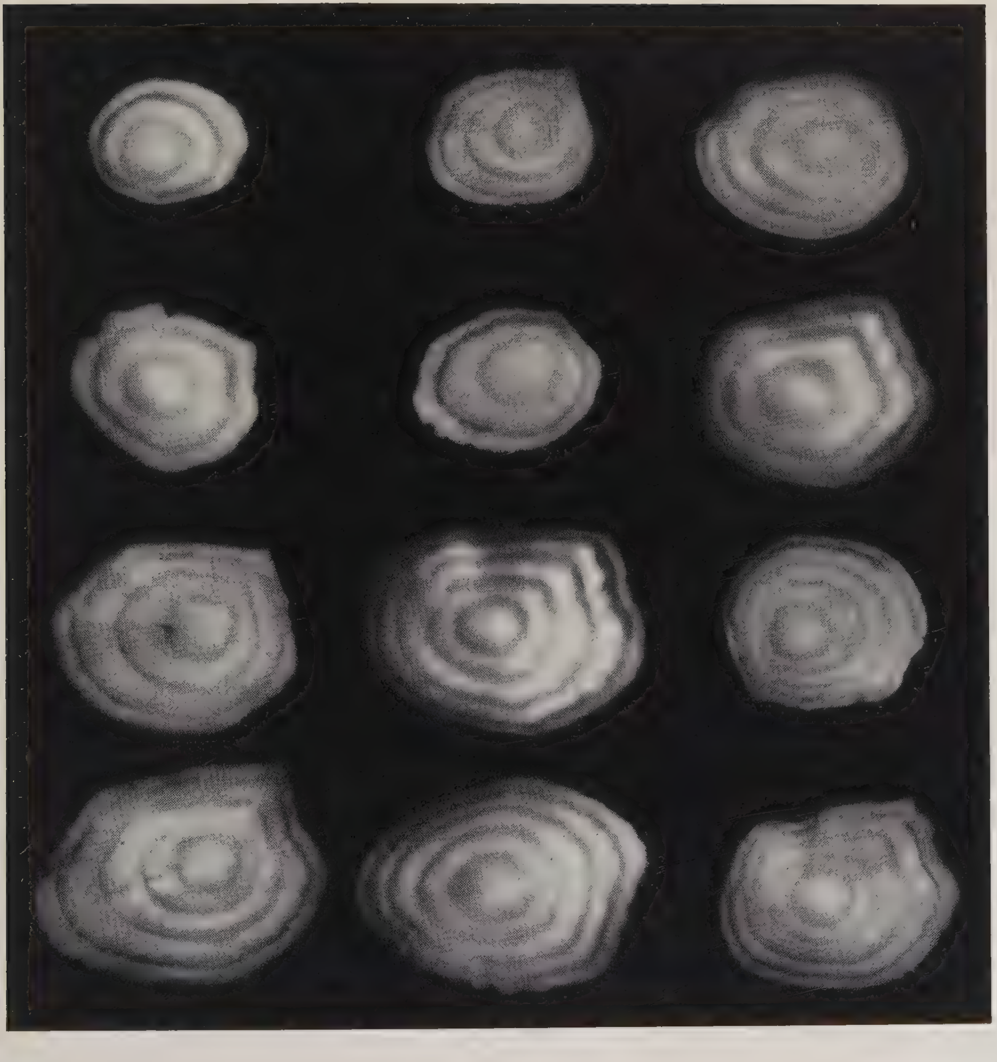


Fig. 6. Growth rate comparison of Clyde and Baltic Long Rough Dabs.

$l_t = 17{\cdot}754\ (1 - 0{\cdot}585^t)$. In the case of the females the observed and calculated values agree quite closely, but with the males there is a greater difference, and it is believed that the discrepancy is due to net selection. The suggestion is made that the calculated mean lengths for each age group are better estimates of those of the population than are the observed sample means. The two standard deviations limits can be estimated about the population means and the sample values are found to be within these limits.

Comparison with previous studies shows that the Clyde fish are smaller and shorter-lived than more northern specimens, though broadly speaking the rate of growth for comparable ages is not so dissimilar.



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EXPLANATION OF PLATE I

Otoliths of Long Rough Dabs. Top row: males left to right: 2 rings, 12·5 cm; 3 rings, 14·0 cm; 4 rings, 19·0 cm. Upper middle row: females, left to right: 2 rings, 17·5 cm; 2 rings, 16·5 cm; 3 rings, 22·0 cm. Lower middle row: females, left to right: 3 rings, 22·5 cm; 4 rings, 23·0 cm; 4 rings, 18·5 cm. Bottom row: females, left to right: 4 rings, 25·0 cm; 4 rings, 23·0 cm; 4 rings, 22·0 cm.

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THE FEEDING BEHAVIOUR OF STARFISH ON ESSEX OYSTER BEDS

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(Plate I and Text-figs. 1–3)

Starfish on Oyster Beds

Two species of starfish are found in large numbers in Essex rivers. They are *Asterias rubens* L. and the common sunstar *Solaster papposus* (L.). Of these, *Asterias rubens* has been regarded for many years as a voracious predator of oysters. Forbes (1841) recorded that it preyed on all kinds of Mollusca, and was commonly found with *Natica* in its stomach. He quotes Bishop Sprat's *History of the Royal Society*, in which the Admiralty Court laid penalties on those engaged in the oyster fishery 'who do not tread under their feet, or throw upon the shore, a fish which they call Five-finger, resembling a spur-rowel, because that fish gets into the oysters when they gape and sucks them out'. Of *Solaster papposus* he said: 'It is very ravenous, devouring shellfish. It frequents oyster and scallop banks, often in great numbers, and sometimes colonizes the sides of harbours frequented by oyster dredgers, in company with *Uraster rubens*.'

It has been readily confirmed since Forbes's time that *Asterias rubens* will feed on oysters, and it has been said to do so with such frequency as to cause very serious depredations. Cuénot (1887) recorded that a natural oyster bed of 10–12 km length had been completely destroyed by starfish, introduced incidentally by fishermen in their nets.

In Canada and the United States starfish present the oystermen with a severe problem. Needler (1941) regarded the starfish (*A. vulgaris*) as the worst enemy of oysters in Canadian Atlantic waters and one of the more serious obstacles to successful oyster farming. Galtsoff & Loosanoff (1939) stated that since the beginning of oyster culture in the United States in 1845, the starfish (*A. forbesi*) has been regarded as one of the most destructive enemies of shellfish on the Atlantic coast, and that by far the greater part of the loss caused is borne by the oyster growers. One estimate is that since 1921 no less than 500,000 bushels of oysters have been destroyed every year, representing an annual loss of 500,000 dollars. The decline in the scallop industry in Buzzards Bay was also attributed to the depredations of the starfish (Galtsoff & Loosanoff, 1939).

Korringa (1951) found that although *A. rubens* occurs on the Dutch oyster beds, the number of adult oysters devoured gives no cause for serious anxiety, because the starfish apparently prefer mussels, or encounter less difficulty in opening them. Serious damage may, however, be caused by young starfishes among the tiny oyster spat on the tile-collectors.

In this country the starfish *A. rubens* has always been regarded by fishermen as the traditional enemy of mussels and oysters. Laver (1916) listed the starfish or 'Five-finger' as the most injurious of the direct enemies of the oyster in the Colchester Oyster Fishery. Collard (1902) referred to the starfish as a deadly foe of the Whitstable oyster.

Large numbers of *A. rubens* appear from time to time on oyster grounds in the River Crouch. They are numerous outside the mouth of the river, and particularly off the coast of Whitstable, where for many years the collection of starfish for fertilizer has been a substantial fishery.

Oystermen in the United States undertake costly control measures, usually with boats equipped with starfish mops. Lee (1951) estimated that the direct cost of control efforts amounts to more than 500,000 dollars annually. Scientists in the United States and Canada have enjoyed considerable success with the use of quicklime as a means of control (Galtsoff & Loosanoff, 1939; Loosanoff & Engle, 1942; Lee, 1951). Particles of quicklime spread over the oyster beds quickly sink to the bottom, and on to the surface membrane of the starfish. The caustic action of slaking lime causes lesions which penetrate and spread, eventually causing the death of the starfish. Its effects on oysters and other commercial species are considered to be negligible.

In Holland, the use of a spiked roller for catching starfish has been tried, but abandoned (Lambert, 1951), because of damage to the oysters. Copper sulphate and lime were found to be effective experimentally as methods of chemical control, but dredging is still the only means of destruction generally employed.

In this country, starfish are removed from the dredges during routine oyster cultivation and killed by drying out.

The work forming the subject of this paper was undertaken to test the efficiency of measures of chemical control of native starfish, and to decide whether more intensive control measures could be justified.

The Food of Starfish

Hunt (1925) examined the stomach contents of *A. rubens* from the Plymouth fishing grounds but found that only a small percentage contained recognizable remains. He rightly concluded that this was a result of their feeding method. The mouth of the starfish is small, and digestion of larger animals is largely external. He however recorded the feeding of *Asterias* on small specimens of the molluscs *Venus*, *Dosinia*, *Mactra*, *Pecten*, *Corbula*, *Cultellus*, *Lutraria*, *Syndosmya* and *Turritella*, and found the remains of the crustaceans *Portunus*, *Diastylis* and *Balanus* and the polychaete *Flabelligera*. In *Solaster papposus* he found occasional remains of *Asterias rubens* and a small *Pecten*, but was unable to induce *Solaster* to feed under laboratory conditions. Blegvad (1914) had previously recorded that *Asterias* is devoured by larger individuals of its own kind and by *Solaster*. According to Mortensen (1927) the food of *Asterias* consists principally of molluscs, but it also eats crustaceans (especially barnacles), worms, echinoderms, even specimens of its own species, indeed almost everything eatable, living or dead. He said that it does considerable damage to oyster cultures, though it would appear that it cannot, by itself, open the large, undamaged oysters. It is itself eaten only by other sea-stars (*Solaster*, *Luidia ciliaris*). Vevers (1949) stated that while still in a young stage *Asterias rubens* eats barnacles and small lamellibranchs, but as an adult it feeds chiefly on worms, crustaceans, other echinoderms and many lamellibranchs, in particular *Chlamys opercularis* (L) and *Mytilus edulis*. In fact it would appear to be omnivorous, eating any living or dead material on which it can get a firm hold. Bull (1934) made some aquarium observations and found that *Solaster papposus*, *S. endeca* and the northern stone crab, *Lithodes maia*, fed in captivity mainly on *Asterias rubens*. *Solaster* also ate numbers of *Metridium senile* but would feed on molluscs or mollusc flesh only under conditions of extreme starvation.

The smallness of the mouth aperture in both *Asterias* and *Solaster* limits the size of prey which can be taken into the stomach in a recognizable form. The mouth aperture of *Solaster* is, however, larger than that of *Asterias*, a *Solaster* of 85 mm maximum radius having an aperture of 17 mm diameter, compared with only 4 mm for the mouth of an *Asterias* of similar size. For this reason recognizable stomach contents are more likely to be found in *Solaster*, but they still represent only the smaller items of its diet. Although the stomach contents of many starfish trawled in the Irish Sea were examined by the author, no *Asterias* and only two *Solaster* contained recognizable remains. Of these, one, of 75 mm maximum radius, contained a small *Chlamys* valve, a *Turritella* shell and several pieces of *Asterias* arm. The second, of 80 mm radius, contained a number of spines which were identical with those of *Psammechinus* taken in the same trawl.

In order to determine the larger food eaten by starfish they must be taken in the field when actually feeding, or observed feeding in aquarium tanks. During the course of dredging in the River Crouch, Essex, it has been established that the largest numbers of *Asterias rubens* are found on and in the neighbourhood of the Southward Laying, 1 mile below Burnham-on-Crouch (Text-fig. 2). Closely associated with it, but in less abundance, is *Solaster papposus*, and almost always in the same dredge are found several stone crabs, *Hyas araneus*. Subsequent dredging in the Rivers Crouch and Colne have confirmed that these three species are usually found together. *Asterias* taken from the dredges are frequently found in a characteristic arched feeding position but usually the prey has been released. Occasionally the prey is retained and has been recorded. *Asterias* from the River Crouch have been observed feeding on oysters, slipper limpets (*Crepidula fornicata*), barnacles, *Spisula* and *Littorina*. *Solaster* has frequently been taken with the arms of *Asterias* in its stomach, and also quite often with pieces of *Alcyonidium gelatinosum*, which is abundant in this river. One small *Gibbula* and a small individual of *Crepidula* have also been found in the stomach of *Solaster* in the field.

The bottom community from which the *Asterias* is taken gives a good guide to the type of food which is being eaten. *Asterias* have frequently been recorded in large numbers on mussel beds off the Kent coast, and outside the mouth of the River Crouch, with obvious evidence of their depredations. Korringa (1951) records that Dutch oystermen occasionally see great numbers of the starfish *Asterias rubens* floating on the surface by means of gas bubbles. They believe that this is a method of migrating to other feeding grounds. In Britain this belief is also held locally, particularly at Whitstable, where the phenomenon is said to be associated with the destruction of one mussel bed and the search for another. It is agreed with Korringa that there is no scientific evidence to support this observation: it may be of abnormal occurrence, or due to pathological conditions, but the reports of local fishermen are seldom without foundation. In the River Crouch, particularly on the Southward Laying, where enormous numbers of *Crepidula* occur farther offshore than the main concentrations of oysters, the *Asterias* were found to be associated with the *Crepidula*, and were found in the dredges to be feeding on it. During 2 weeks in February and March, more than 10,000 *Asterias* were removed by the Ministry's two vessels from the offshore part of the Southward Laying, where the fauna consists almost entirely of *Crepidula* with some barnacles. In the mouth of the River Colne recent changes in conditions have led to the almost complete disappearance of oysters. *Crepidula* are abundant there in places, and also *Asterias*, which are often taken in the dredges feeding on *Crepidula*. Mr Francis, foreman of the Colne Fishery Board, was so convinced that the *Asterias* were substantially reducing the population of *Crepidula*, that, contrary to normal practice the starfish were returned to the ground to continue their good work.

Confirmation of the feeding behaviour suggested by field observations was sought in laboratory experiments. First, it was demonstrated that quicklime, sprinkled over the surface of the water in porcelain sinks containing *Asterias*, caused severe burns and lesions on the starfish, followed by their disintegration and death within about 3 days. Attention was then directed towards the advisability of using such drastic control measures on the oyster beds. It was decided that a series of laboratory experiments would help to assess the magnitude of the predations of starfish on the oysters.

Laboratory Observations

During the course of the experiments a large stock tank containing various shellfish and starfish was maintained, and provided a series of interesting observations. The tank contained a population of mixed sizes of *Asterias rubens* with several *Solaster papposus* and *Hyas araneus*, and an abundance of oysters and oyster spat, barnacles, *Urosalpinx* and *Crepidula*. During the period 11–27 January 1954, although many *Crepidula* and some *Urosalpinx* and barnacles had been devoured, only one small oyster and no oyster spat had been eaten. Between 17 March and 21 April 1954, a total of 262 *Crepidula*, and 28 *Urosalpinx* were devoured compared with only two brood oysters and no oyster spat. No count was kept of barnacles eaten. On one occasion, the introduction of a quantity of fresh *Crepidula* chains into the corner of the stock tank was followed almost immediately by the migration of thirteen out of twenty of the *Asterias* to that corner and extensive feeding.

Other shellfish devoured by *Asterias* in this stock tank included several *Pecten maximus* and *Mya arenaria*. One *Asterias* was observed attacking and devouring a smaller individual of the same species.

During the course of these observations *Solaster* was once observed feeding on a brood oyster, and very occasionally on a *Crepidula* chain. It was frequently observed to attack *Asterias*, individual arms of which were often taken from the stomach of *Solaster*.

Asterias, but rarely *Solaster*, was frequently attacked by *Hyas* (see 'Method of feeding' p. 321), which injures the arms by constricting them with its chelae, and biting the tips with its mandibles. A check on the *Asterias* in the tank showed that six *Solaster* and six *Hyas* had between them accounted for several complete *Asterias*, between one and three arms from each of nine others, and the tips or ends of arms from eight others during a period of 2 weeks in March. The *Solaster* had only rarely been damaged.

Several shore crabs, *Carcinas maenas*, which are found in the dredges with *Hyas*, were introduced into the tank, but on no occasion were they suspected of attacking starfish.

Further experiments were designed to discover the type of food on which *Asterias* and *Solaster* will feed. These were followed by offering *Asterias* various foods in equal quantity, in order to determine those which are the most important in its diet.

Experiment 1

This was designed to estimate the rate of destruction of brood oysters in the absence of other food.

Four large *Asterias* (maximum radius 140 mm) were placed in a large tank containing sixteen adult oysters. As each oyster was consumed it was removed, and replaced by another of approximately the same size. It was found that the *Asterias* assumed a feeding position in less than 2 days, and within 3 days of commencing the experiment two oysters had been consumed. During the period 7 January–21 April 1954, the four starfish consumed an average of 4·8 oysters per week. The largest number of oysters eaten in 1 week was eight. The oysters used were between 44 and 76 mm average diameter. One *Asterias* (♂) spawned on 11 May at a temperature of 15·3° C. Two others spawned on 29 May. Feeding stopped a few days before and after spawning and did not resume its former intensity throughout the summer. Generally an oyster was opened and consumed in less than 2 days. One starfish was observed to complete the process in 22 h during March, and a period of less than 24 h was recorded on several occasions. Evidently the larger starfish can and will eat oysters consistently in the absence of other food, and their rate of feeding under such conditions would constitute a serious menace to oyster growers.

Experiment 2

This was designed to discover on which other animals the starfish will feed, in the presence of oysters. Four *Asterias* ranging from 50 to 120 mm maximum radius of arm, were confined with abundant food including oysters, oyster spat, *Mytilus*, *Cardium*, *Paphia*, *Macoma*, *Buccinum*, *Crepidula*, *Nassarius*, *Nucella*, *Urosalpinx*, *Littorina*, *Gibbula* and barnacles. It was not possible to keep equal numbers of each food throughout the experiment or to replace each one immediately it was consumed, but a stock of 6 oysters and 6 shells bearing oyster spat (17) was maintained, and at no time did the total of any other species exceed this figure. From 4 January to 24 March 1954, the 4 *Asterias* consumed 45 *Mytilus*, 12 *Macoma*, 10 *Cardium*, 4 *Urosalpinx*, 4 *Nucella*, 3 *Paphia*, 3 *Crepidula*, 1 *Nassarius*, 1 *Littorina* and 1 *Gibbula*. It is significant that during this time no oysters or oyster spat were consumed. It was concluded that barnacles provided little attraction because many of the food animals devoured were coated with undamaged barnacles, mainly *Elminius*.

Experiment 3

Four *Solaster* (55–70 mm maximum radius) were confined with abundant food supply including oysters, oyster spat, *Mytilus*, *Cardium*, *Littorina*, *Urosalpinx*, *Crepidula*, *Macoma*, *Metridium* and 1 *Psammechinus*, but no *Asterias*. From 5 January to 11 March, only 3 *Cardium*, 2 *Macoma*, 1 *Psammechinus*, 1 oyster spat and 1 *Metridium* were devoured. It has been found that the feeding rate of starfish decreases before and during spawning. The *Solaster* spawned in the sink on 25 February, but this low rate of feeding could not entirely be associated with this fact, but was almost certainly due to an unsuitable food supply. When, on 12 March, an *Asterias* was added it was rapidly devoured, the remains being found in the stomachs of 3 *Solaster*.

When further *Asterias* were added they were attacked almost at once by the *Solaster*. Evidently *Solaster* can eat other food, but its natural food certainly includes *Asterias*, confirming the observations made in the field.

Experiment 4

The preliminary experiments seemed to indicate that *Mytilus* provides the greatest attraction to *Asterias*, but the number of mussels occurring at the Southward Laying (Mistakidis, 1951, and own observations), and generally in the River Crouch, is insignificant compared with other organisms. For this reason, only the more important animals related to oyster cultivation were included as food in this experiment. Four *Asterias* (maximum radius 60–120 mm) were confined with 6 brood oysters, 6 chains of *Crepidula*, 6 oyster shells each bearing several oyster spat, and 6 *Urosalpinx*. Between 25 March and 22 June, 1 brood oyster, 8 oyster spat, 27 *Crepidula* and 10 *Urosalpinx* were devoured.

Experiment 5

During the field observations mentioned previously, it was found that *Asterias* is frequently found feeding on *Crepidula*. In order to determine to what extent *Asterias* will feed on *Crepidula* in the presence of oysters, 10 *Asterias* (maximum radius 70–108 mm) were confined with 20 *Crepidula* chains and 20 small oysters, the largest oyster measuring 54 mm average diameter. These were distributed at random in a large tank with running sea water. After 5 days (20–25 March 1954) it was found that every *Crepidula* chain had been attacked to some extent, with as many as five individuals eaten from one chain. A total of 53 individuals of *Crepidula*, most of them adult, were eaten. This must be compared with the consumption of only 3 oysters. In a subsequent experiment the same 10 *Asterias* were used and 20 adult individuals of *Crepidula* were distributed at random with 20 oysters. During a period of 14 days (25 March–8 April) 9 oysters and 13 *Crepidula* were eaten. The possible explanations of the lower proportion of *Crepidula* eaten are believed to be first, that a chain of individuals exerts a greater attraction than a single one, or, secondly, that, attraction being discounted, the random movements of the starfish were interrupted by the presence in one place of a series of readily accessible animals provided by a chain. It must be remembered, however, that in the first part of this experiment all 20 of the chains had been attacked by *Asterias*, and that under natural conditions there is an ample supply of *Crepidula* which are more likely than not to be concentrated in chains. In a subsequent experiment 20 oysters were placed in one corner of the tank (area 160 cm square) and 20 chains of *Crepidula* in the opposite corner, with 10 *Asterias* in the centre of the tank. In 6 days (8–14 April), 4 oysters and 33 *Crepidula* were eaten. The experiment was repeated, reversing the positions of *Crepidula* and oysters. In the subsequent 6 days, 4 oysters and 25 *Crepidula* were consumed. This behaviour confirms the suggestion that *Asterias* prefers *Crepidula*, possibly because it is less inaccessible than the oyster.

Experiment 6

It became evident from observations made in the stock tank and subsequent experiments that there was a difference in feeding behaviour according to the size of the starfish. A population of *Asterias* was divided arbitrarily into size-groups by reference to a histogram composed from measurements of *Asterias* dredged from the River Crouch (Text-fig. 1). The groups selected were—group I: < 30 mm; group II: 40–70 mm; group III: 70–100 mm; group IV: > 100 mm maximum radius.

Group I. 23 *Asterias* of maximum radius 30 mm were confined with shells and stones bearing oyster spat, *Crepidula* spat and barnacles, and 6 *Urosalpinx*. The tanks were examined every day or two and the food eaten was replaced. During the period 22 April–10 May 1954, a total of 15 oyster spat, 13 *Crepidula* spat and 6000 barnacles, mainly *Elminius*, had been devoured. These results were consistent throughout the experiment and it was significant that on several occasions an oyster shell was cleared of barnacles leaving several undamaged oyster spat. Further, it was found that the number of oyster spat devoured was always greatest when virtually all the barnacles had been devoured.

Group II. 10 *Asterias* of maximum radius 40–70 mm were confined with 6 young oysters, 6 shells bearing oyster and *Crepidula* spat, 6 chains of *Crepidula*, 6 *Urosalpinx* and 2 stones covered by barnacles. During the period 22 April–10 May 1954, a total of 1 oyster spat, 2 *Crepidula* spat and 3500 barnacles were consumed. Although no selection was made of barnacles used in the experiments, those eaten by group II were mainly of a larger size than those eaten by group I, and it was found that the larger barnacles were eaten before the smaller sizes were attacked.

Group III. 7 *Asterias* of maximum radius 70–100 mm were confined with 6 young oysters, 6 shells bearing oyster and *Crepidula* spat and barnacles, 6 chains of *Crepidula*, 6 *Urosalpinx* and 2 stones covered by barnacles. Between 22 April and 10 May 1954, a total of 3 oyster spat, 13 *Crepidula* spat, 2 oysters, 47 adult *Crepidula*, 1 *Urosalpinx* and 425 barnacles were devoured.

Group IV. 5 *Asterias* of maximum radius 100–145 mm were confined with unlimited quantities of oysters and oyster spat, *Crepidula* and its spat, *Urosalpinx* and barnacle covered shells and stones. During the period 22 April to 14 May 1954, a total of 1 oyster, 29 adult *Crepidula* and 20 barnacles together with 1 *Pecten maximus*, which was being stored in the same tank, were eaten. This confirms the previous result (Exp. 2) that the barnacles provide little attraction for the larger starfish.

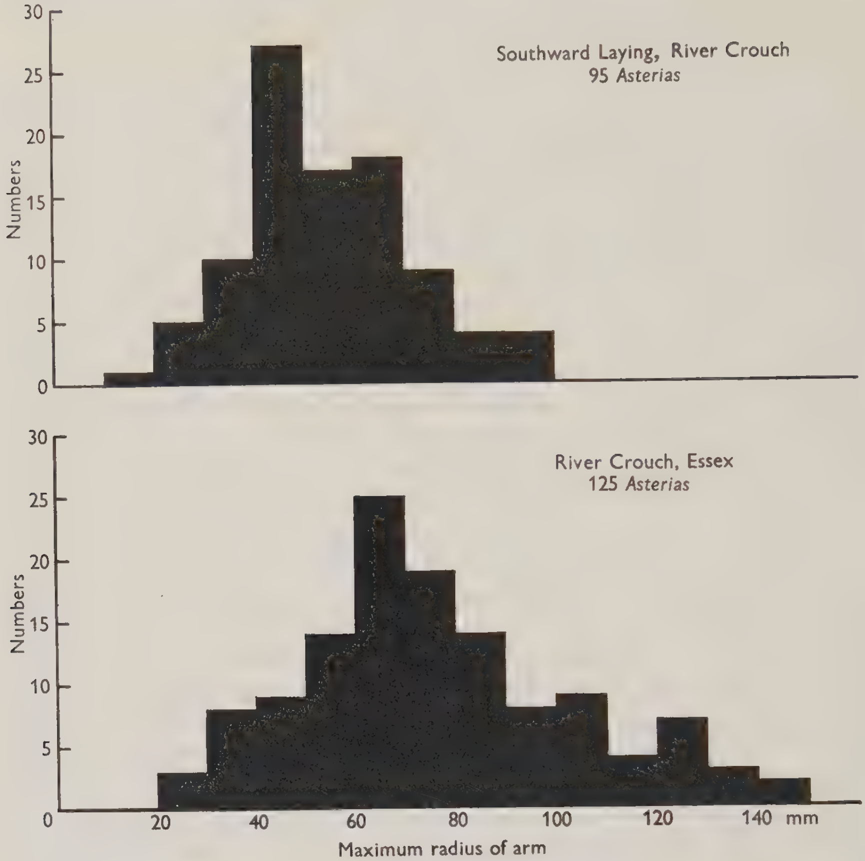
In Table I, a comparison is made of the average numbers of different foods eaten by one *Asterias* from each group in 18 days. It can be seen that the smaller sizes of *Asterias* ate mainly barnacles, with only occasional oyster or *Crepidula* spat. The larger sizes took fewer barnacles, and ate significantly more *Crepidula* and its spat than oysters and oyster spat.

It can be seen by reference to the histogram composed from measurements of *Asterias* dredged from the River Crouch on 10 May (Text-fig. 1) that groups II and III formed the most substantial proportion of the starfish population. The largest proportion of starfish dredged from the Southward Laying (Text-fig. 1) was in group II. The starfish in this histogram are representative of the total population at the Southward Laying, since all parts of the oyster ground were sampled by dredge. Using the histogram and the results shown in Table I, the numbers of oysters and their competitors which would have been eaten by the 95 starfish during 18 days in April–May have been calculated as: 24 oyster spat, 54 *Crepidula* spat, 5 oysters, 114 *Crepidula*, 2 *Urosalpinx* and 26,900 barnacles. This gives a clearer picture of the proportions of food organisms which could be expected to be devoured by the natural starfish population of the Southward Laying under experimental conditions.

METHOD OF FEEDING

Little new information is added here on the actual means by which *Asterias* and *Solaster* open the shells of their molluscan prey. The starfish wraps its arms round its prey, attaches itself by the series of tube feet, and proceeds to pull the valves apart. Once opened, the stomach of the starfish is extruded (Pl. I, fig. 1) into the prey and digestion commences. Extrusion of the stomach is brought about by contraction of the oral and aboral surfaces of the animal which forces coelomic fluid into loose separate folds of the cardiac stomach (Cuénot, 1887). Irving (1924) worked out the ciliary currents in the starfish *Patiria*, and decided that digestion products are distributed by ciliary action, the Tiedemann's diverticulum being a region specialized for distribution.

The mechanics of the process by which the prey is opened have provided a subject for controversy for many years. Schiemenz (1895) explored most possibilities and concluded that a starfish can develop sufficient force to open a bivalve unaided by any poisonous secretion. Van der Heyde (1922) established the presence of a toxic substance in the stomach extract of the starfish *Asterias forbesi*, using the heart of *Pecten* and the gastrocnemius muscle of a frog. Sawano & Mitsugi (1932) found that the stomach extract of *Asterias rollestoni* caused a tetanic contraction in the heart of the oyster. Cahn (1950) has described the feeding process in the Japanese starfish (*A. amurensis*), which begins its attack on the oyster by pouring a poison secreted by its stomach into the water in front of the oyster's inhalant siphon. The poison in the inhalant current of water stimulates the oyster to close its shell. The adductor muscle loses tonus and relaxes, and the valves are pulled apart by



Text-fig. 1. Population histograms of *Asterias rubens* from a dredge survey on 10 May 1954.

TABLE I. SHELLFISH DEVOURED BY *ASTERIAS* DURING THE INCLUSIVE PERIOD 23 APRIL–10 MAY 1954

Figures reduced to 1 *Asterias* in each size group

Size group ...	I	II	III	IV
Max. radius (mm) ...	15–30	40–70	70–100	100–145
Oyster spat	0·7	0·1	0·4	0
Crepidula spat	0·6	0·2	1·9	0
Oysters	0	0	0·3	0·2
Crepidula	0	0	6·7	4·7
Urosalpinx	0	0	0·1	0
Barnacles	260	350	61	3

the tube feet of the starfish. The time required to destroy an oyster is about 2 h from the first injection of the poison into the water to the complete digestion of the body. Experimentally, this paralysis remains effective for many hours, if it does not result in death.

Even if such an effect could be produced by the stomach extract of *A. rubens*, this explanation is still open to Schiemenz's criticism that a bivalve which has been opened but not digested will quickly recover. With oysters, mussels and *Pecten*, the author has observed on several occasions that the disengaging of the arms of the starfish has resulted in such rapid closure by the bivalve that the stomach of the starfish was trapped and torn. Evidently if *A. rubens* is able to produce a poison, it can only have a temporary effect and appears not to be lethal.

It is interesting that the outer margin of the oyster shell is invariably broken away during an attack by *Asterias*, but in other bivalves and *Crepidula* the shell margin remains intact. This could be said to result from a deliberate chipping away of the edge, which could allow the entry of a narcotic, but is more likely to be due to a strong pull by the starfish which causes the weakest part of the shell to break away first. This conclusion was strengthened when an *Asterias* attacked an oyster which had been suffering from a severe attack by *Polydora*, the shell-boring mudworm. Here the edge of the shell remained unbroken, while one valve was broken across where it had been weakened by the excavation of *Polydora* in the region of attachment of the adductor muscle.

A chain of *Crepidula* may be attacked from either end or at some intermediate point in the chain. Once an individual has been detached or the chain parted the diverticula of the stomach are everted around the now exposed body of its prey.

The barnacle is the favourite food of the smaller sizes of *Asterias*. *Elminius modestus*, the dominant barnacle in the bottom community, is eaten most frequently, but also *Balanus* where it and *Elminius* occur together. The method of feeding appears to depend on the strength of the starfish. The group I starfish usually ate all the barnacles which they were capable of detaching, by sucking the contents through the underside. The larger barnacles were then devoured by sucking the contents through the operculum. The group II starfish could feed on all sizes of barnacles by detaching them, but the smaller barnacles were not usually attacked until the larger ones had been eaten.

Solaster seldom overcomes a complete *Asterias* at once. An *Asterias* in captivity is actively pursued by *Solaster* which attaches itself to one or more arms of the *Asterias*. An arm is soon autotomized by the *Asterias*, and devoured by the *Solaster* (Pl. I, fig. 1). After digestion, the spicules of *Asterias* are rejected.

Hyas presents an interesting study in behaviour. When an *Asterias* is feeding on a mollusc, the *Hyas* appears to be waiting for scraps, and when the shell of the prey is discarded it claws out what few remains the *Asterias* has left. In many instances, however, the *Hyas* becomes impatient and attacks the *Asterias* itself, which it grips between its two chelipeds then proceeding to use its mandibles to chew off the tip of the arm of the *Asterias* (Pl. I, fig. 2). Since the sensitive eye spot is located here such an action must cause serious damage to the starfish, but in many cases it is followed by complete autotomization of the arm, which is then devoured. In some instances the constriction of the arm by the chelipeds of *Hyas* is so severe that the distal portion of the arm becomes functionless and withers. In one of the specimens in Pl. I, fig. 2, a new arm tip appeared at the point of constriction after several weeks. It is a strange fact that although *Hyas* frequently attacks and feeds on *Asterias* it seldom attacks *Solaster*. This may be coupled with an interesting local tradition that *Solaster* may be used as bait to poison marauding garden cats, but *Asterias* is not mentioned in this capacity. It is possible that *Hyas* can detect some toxic substance in the flesh of *Solaster* and it was decided to investigate this by simple experiment. Ten small pieces, from each of a freshly dead *Asterias* and *Solaster*, were placed in a glass trough with two *Hyas*. After several hours it was found that all the pieces of *Asterias* had been consumed, while all ten of the pieces of *Solaster* remained. After another hour, no further feeding had taken place, and ten more pieces of *Asterias* were added with the same result. Evidently there is some substance in *Solaster* which renders it undesirable to *Hyas*. I have, however, observed one *Solaster* eat a smaller one of the same species, but it was almost certainly already dead.

RATE OF FEEDING

Experiments in Canada (Needler, 1941) showed that one starfish may kill three oysters of about half its own length in one week, and much larger numbers of spat. A starfish was found by experiment to require a diameter of about 1½ times the length of an oyster to attack it successfully. Since most of the starfish on Canadian beds are between 2 and 3 in. in diameter, the greatest danger is to spat or small oysters.

In America Mead (1901) noticed that a single small *Asterias forbesi* devoured over 50 clams (*Mulinia lateralis*) in 6 days. Galtsoff & Loosanoff (1939) showed in the laboratory, at Milford, Conn., that one medium-sized starfish consumed thirteen 1-year-old oysters in 4 days. One small starfish of 1·7 cm diameter destroyed 25 oyster spat of less than 1 cm diameter in 3 days. They found that in experiments conducted in outdoor tanks during the pre-spawning period from May to July, the starfish fed only rarely, but after completion of spawning they became exceedingly voracious until the onset of low temperatures in winter and early spring.

In Exp. 1 (above) the starfish were confined in indoor tanks protected from the influence of lowest temperatures. Uninterrupted feeding took place from the beginning of January to the beginning of February, although temperatures dropped at times to a minimum of 3·0° C. Feeding ceased completely between 3 and 12 February when minimum temperatures of 2·0° C. were recorded, and was resumed as temperatures began to rise again. One male *Asterias* spawned on 11 May and 2 others on 29 May, but feeding was interrupted only for a few days before and after spawning, although the *Asterias* were greatly swollen with gonads. In this instance the behaviour of *A. rubens* seems to differ slightly from *A. forbesi*, which is inhibited from feeding by temperatures between 0 and 6° C. The rate of feeding during the summer months was less than that during the first 4–5 months of the year. Although an oyster was devoured in less than 24 h, the maximum number eaten by four starfish in one week during the period under observation was eight, representing an average of two per starfish. These oysters were at least 3 years of age and between 44 and 76 mm average diameter.

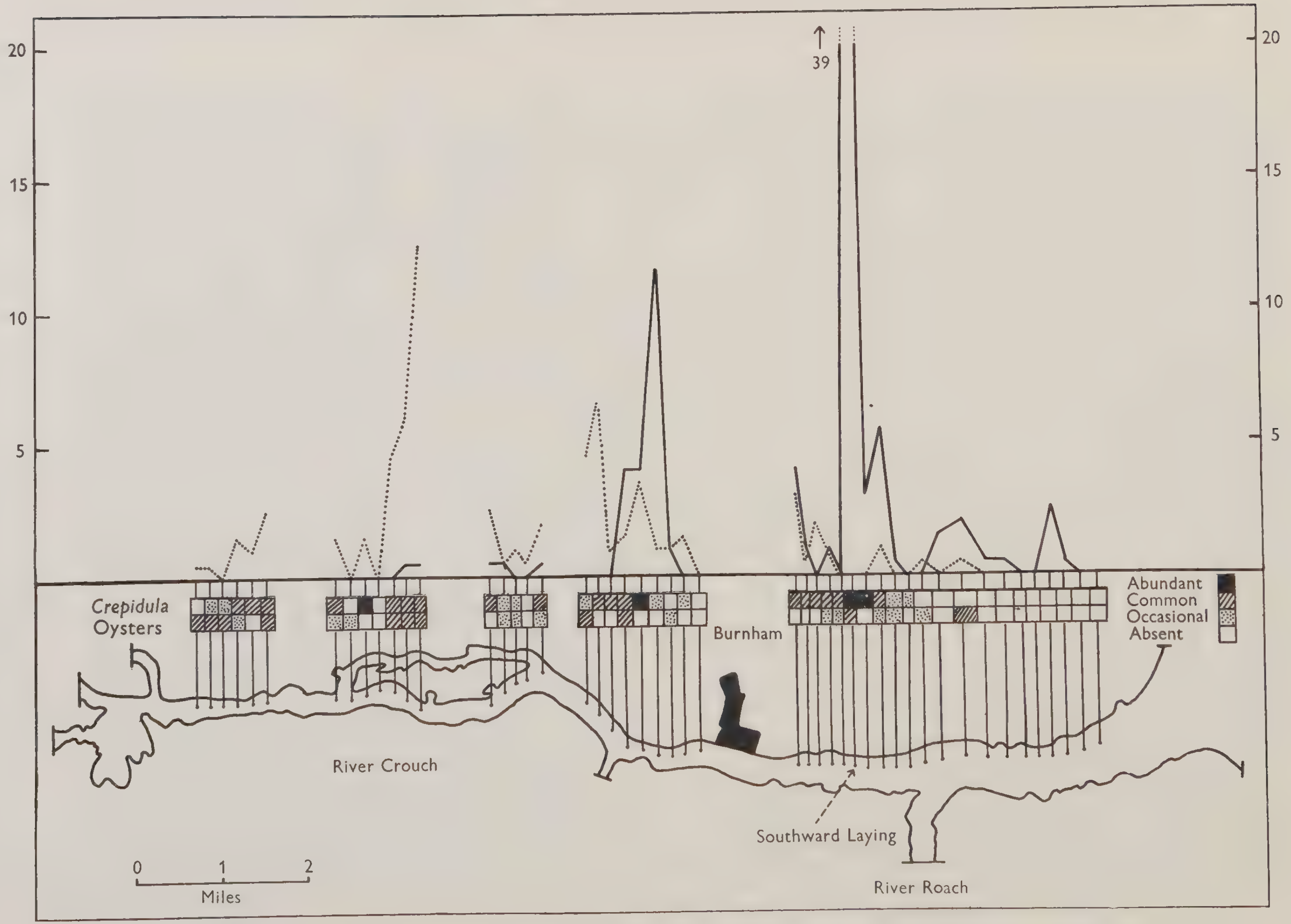
Subsequent experiments showed that the smaller sizes of *Asterias* ate up to 200 barnacles each in one week. Larger starfish ate two to three adult mussels per week, each one usually taking between 15 and 24 h to open and consume one mussel.

In the feeding experiments an *Asterias* from group III ate significantly more than a larger starfish from group IV in the same time.

Feeding and Distribution

The distribution of *Asterias* and *Solaster* in the River Crouch was investigated by a survey on 10 May 1954. Five-minute dredge hauls were made, using two 4 ft. power dredges, throughout the length of the river along a line intermediate between L.W.O.S.T. and mid-river. It is known that the numbers of starfish vary in relation to the distance offshore and the type of bottom, and this arbitrary line was selected as being most likely to reveal the presence of both species. The results have been interpreted graphically (Text-fig. 2). Certain stretches of the river could not be sampled, usually because of the presence of large numbers of boat moorings.

Both *Asterias* and *Solaster* were absent from the lower end of the river and this is believed to be due to the presence of accumulations of silt, since both species are found just outside the river, where *Asterias* in particular is very abundant. The largest numbers of *Asterias* were found in the neighbourhood of Burnham-on-Crouch, with the greatest concentration at the Southward Laying. The numbers of *Asterias* fell markedly higher upstream and none were found more than 4½ miles above Burnham. *Solaster*, however, was found to be present throughout the river at least as far as 7 miles above Burnham, beyond which no further samples were taken. The largest numbers of *Solaster* were found higher upstream than the main concentrations of *Asterias*. It was evident from the results that the largest numbers of *Asterias* were associated



Text-fig. 2. Distribution of *Asterias* and *Solaster* in the River Crouch, Essex, and the density of *Crepidula* and oysters.

with derelict or semi-cultivated oyster ground with a bottom fauna characterized by a high proportion of *Crepidula*. *Asterias*, and less frequently *Solaster*, are sometimes found on the shore above L.W.O.S.T.

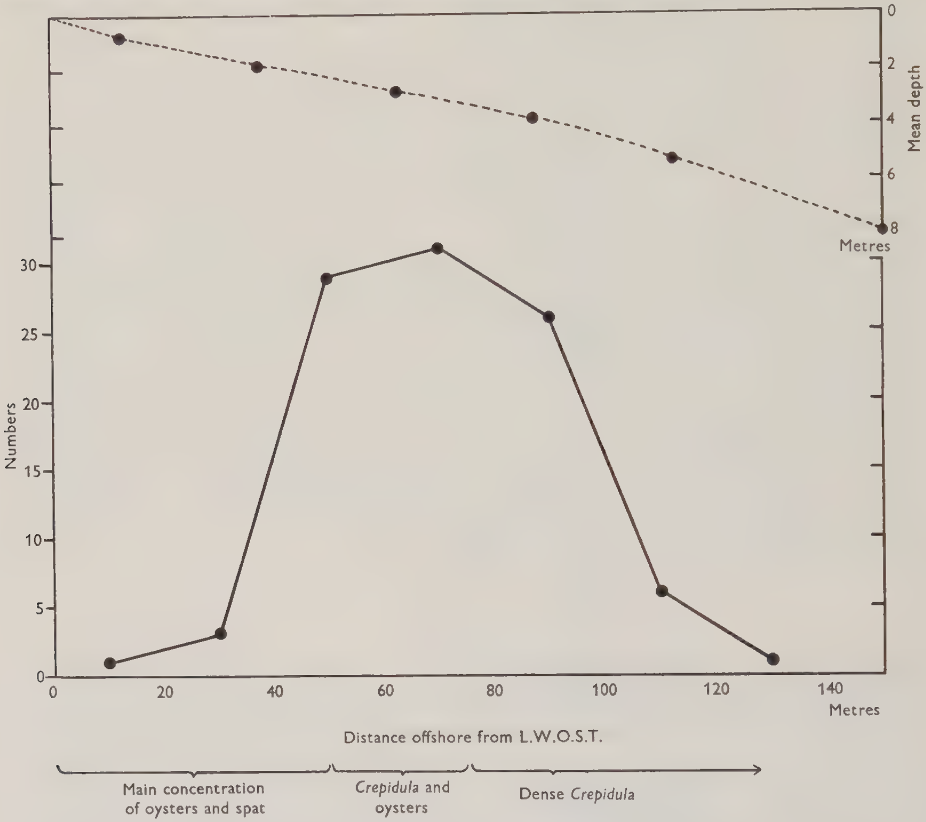
A more intensive survey was made of the distribution of starfish over the Southward Laying, where the highest concentration of *Asterias* was recorded. This is an oyster ground which has the characteristics of both cultivated and uncultivated ground in its different parts. The shore is mainly muddy, and *Asterias* is only infrequently found above L.W.O.S.T. Previous surveys made using a van Veen grab sampler had shown that the main concentrations of oysters and their spat occur up to 50 m offshore, largely as a result of intensive cultivation of this area and removal of *Crepidula*. Further offshore there are proportionately more *Crepidula*, until by about 75 m from low-water mark the bottom fauna consists almost entirely of chains of *Crepidula*. Barnacles are profuse over most of the area, their numbers dropping slightly further offshore. A dredge survey was made of this laying by taking parallel hauls with two oyster dredges at 20 m intervals. The first haul was made parallel to the shore and 10 m offshore from L.W.O.S.T. It was found (Text-fig. 3), that the main concentrations of *Asterias* occur on the offshore part of the ground in a region characterized by a high population of *Crepidula*. It was from this area that the 10,000 *Asterias* mentioned previously had been removed in 3 weeks' dredging in March. The River Crouch is 450 m wide in the Southward Laying region. It can be seen that *Asterias* occupies the area between L.W.O.S.T. and about 150 m offshore. *Solaster* is more likely to be found in the mid-river part, overlapping the *Asterias* range only slightly.

DISCUSSION

Ecologically, the various rivers of Essex provide very similar situations, and *Crepidula* forms the bulk of the benthic biomass (Knight-Jones, 1952). Mistakidis (1951) found that in the Rivers Crouch and Roach the climax community on uncultivated ground is dominated by *Crepidula*, and in the River Blackwater by *Crepidula*, *Ascidiella* and *Ciona*. On cultivated well-stocked oyster grounds where *Crepidula* is present the equilibrium is an artificial one and has to be maintained by frequent dredging and harrowing. On such oyster beds the bottom community in Essex may be described as characterized by *Ostrea* with *Elminius* as epifauna. *Urosalpinx* occurs in varying numbers in the Essex rivers and is well suited by the conditions on cultivated grounds.

It is evident that there are ample quantities of food for starfish in the rivers, where cultivated and uncultivated grounds occur side by side. Although *Mytilus* is not abundant in the River Crouch, it appears to provide the favourite food of *Asterias*. *Mytilus* is usually discouraged on oyster beds because of its habit of binding oysters and shells bearing spat together with byssus threads and because its presence increases the deposit of silt.

During the laboratory experiments it was noted that when oyster spat was devoured by *Asterias* the two valves were only rarely detached from each other. At the end of 1953, a survey had been made of the Southward Laying, Essex, using dredges and a van Veen grab sampler. From the results of this survey it was calculated that there had been a total mortality of 73% of the 1953 settlement of oyster spat before the end of December 1953. It was estimated that 58% of the spat had been destroyed by *Urosalpinx*, leaving a mortality of only 15% attributable to other causes (Hancock, 1954). One of these causes is likely to be starfish, although smothering by silt and attacks by *Carcinus* are considered to be prominent amongst the dangers to which oyster spat are exposed (Knight-Jones, 1952). It can be concluded that *Asterias* is by no means the most important cause of mortality amongst oyster spat in these rivers, although it may account for some of it.



Text-fig. 3. Distribution of *Asterias rubens* on the Southward Laying, River Crouch.

From the viewpoint of oyster culture, the problem is to what extent *Asterias* behaves as a predator on oysters and their spat. There is evidence from observations in the field that the largest numbers of *Asterias* are to be found amongst the most dense concentrations of *Crepidula*. Laboratory experiments have shown that when oysters and *Crepidula* are present side by side, larger numbers of *Crepidula* are likely to be consumed. The smaller sizes of *Asterias* show a marked preference for barnacles over oyster spat. *Crepidula* and barnacles occur in enormous numbers in the River Crouch and compete with the oyster and its spat for space and food supply. *Asterias* often feeds on *Urosalpinx*, which has been shown to eat large numbers of oyster spat. It would not be possible to estimate the negative value of pests eaten by starfish in terms of oysters, but *Asterias* has been found to feed significantly more on animals which are important predators and competitors than on the oysters themselves. The figures from the feeding experiments show that *Asterias* must play a large part in reducing their numbers. They also show, however, that the larger starfish can and will eat many oysters in the absence of other food. In Essex, oyster grounds are seldom free from large numbers of barnacles or remote from quantities of *Crepidula*. It must be concluded that *Asterias* provides some measure of control of the pests of oyster grounds and as such can be regarded as beneficial to oyster culture. There is no doubt that a certain number of oysters and spat fall prey to *Asterias*, but it is felt that severe damage is only likely to occur when starfish are present on highly cultivated grounds with large concentrations of oysters and their spat. In such circumstances, removal and destruction of the starfish is recommended. When, however, a derelict ground is being cleaned by dredging and any *Asterias* taken can only be feeding on *Crepidula* and barnacles, to destroy the starfish would be a misguided policy. Starfish are known to be capable of migrating, but there has been no evidence that under the conditions in these rivers a cultivated ground provides a greater attraction than an uncultivated one. It is possible that under controlled conditions starfish could be transplanted to the more isolated of the derelict grounds and used as a means of reducing the numbers of pests. Before relaying oysters to such a ground, chemical control to remove the starfish might have some application, but it is felt not to be justified under normal conditions.

Solaster and *Hyas* have been found to be natural enemies of *Asterias* and must have some effect in limiting the size of its population. The two species of starfish differ slightly in distribution, and this fact may lessen the effects of feeding by *Solaster* on *Asterias*.

SUMMARY

Laboratory and field observations were made on the food and feeding of the starfish in relation to its role as a predator on oyster beds in the rivers of Essex, particularly in the river Crouch.

Asterias rubens was found most likely to be associated with large numbers of *Crepidula*, the most serious competitor of the oyster.

Laboratory experiments showed that although *Asterias* occasionally ate spat and adult oysters, the greater part of its food was made up of organisms which are competitors of the oyster. The smaller sizes of *Asterias* ate large numbers of barnacles, with occasional spat of oysters and *Crepidula.* The larger occasionally ate oysters and oyster spat, but almost always exhibited a preference for mussels and, in the absence of these, for *Crepidula,* and sometimes even for *Urosalpinx.*

Some observations were made on the method and rate of feeding and distribution of *Asterias* and *Solaster papposus,* and certain aspects of the feeding behaviour of the stone crab, *Hyas araneus.*

It was concluded that *Asterias* is not such a serious enemy of the oyster as was previously supposed, and that under certain conditions, its presence may be beneficial to oyster culture.

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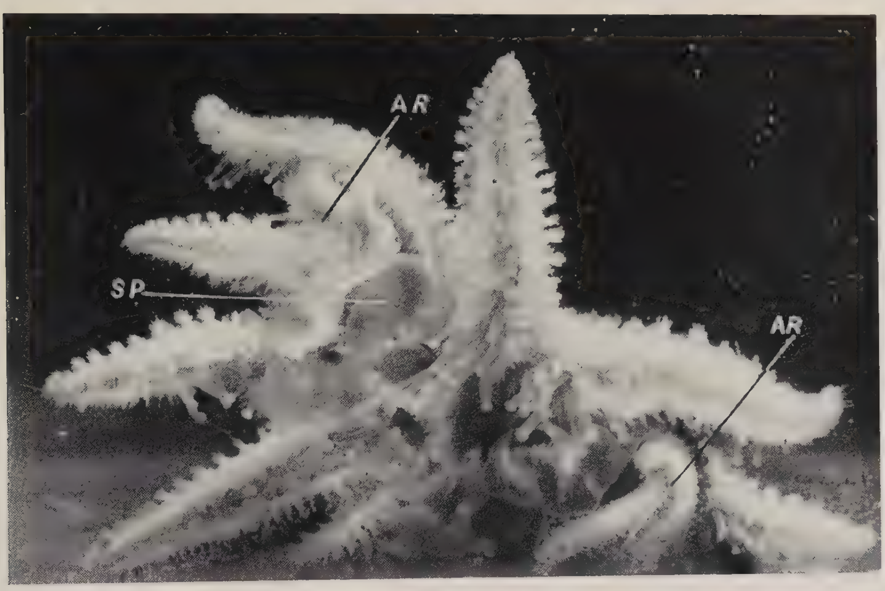

Fig. 1

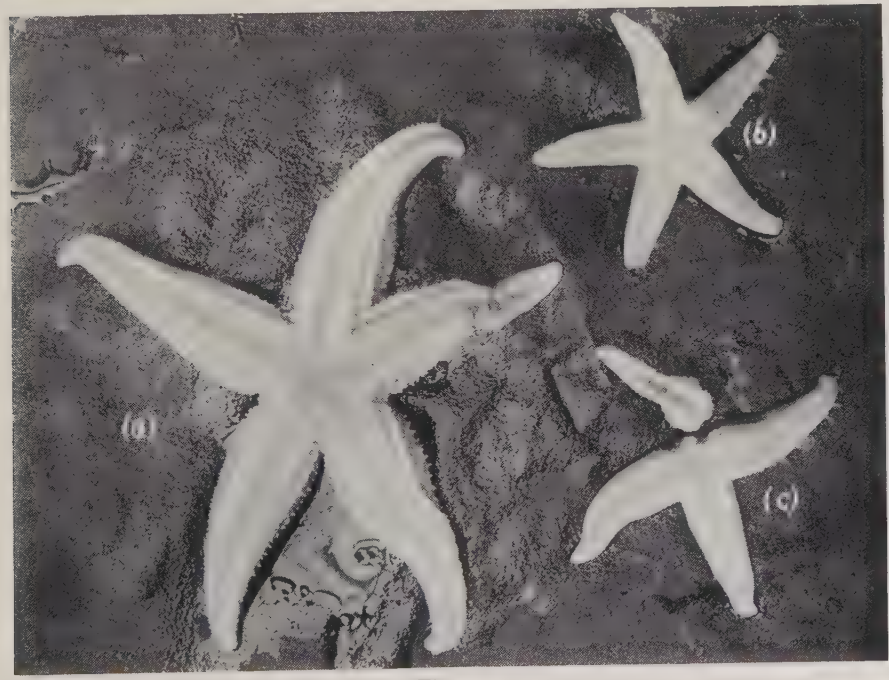

Fig. 2

(*Facing p.* 330)

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EXPLANATION OF PLATE I

Fig. 1. *Solaster papposus* attacking *Asterias rubens*, which has autotomized an arm. *AR.*, arms of *Asterias*; *SP.*, everted stomach pouches of *Solaster*. Photo: P. J. Warren.

Fig. 2. *Asterias rubens* attacked by *Hyas araneus*, showing (*a*) constriction of one arm, (*b*) removal of three arm tips, and (*c*) autotomization of two arms. Photo: D. Key.

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NOTES ON THE VARIATION OCCURRING IN *TUBULARIA LARYNX* ELLIS & SOLANDER

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(Text-figs. 1–5)

In the course of an investigation into the settlement of actinula larva of *Tubularia larynx* Ellis & Solander, the variations in the material collected suggested that more than one species of *Tubularia* might be present at Plymouth. Pyefinch & Downing (1949) had noted variations in the species occurring at Millport, but had decided that it could most satisfactorily be included in *T. larynx*.

Brink (1925*a*) drew attention to Fenchel (1905), who showed that twenty-two forms of *Tubularia* described as new species were *T. larynx*. Amongst these Fenchel included Hincks's (1868) *T. coronata* and Allman's (1871) species *spectabilis*, *pacifica*, *attenuata*, *polycarpa*, *tenella*, *bellis* and *humilis*. These had been given status as species because insufficient notice had been given to the variability of the specific characters.

Errors have also been made because immature individuals have been described. Brink (1925*b*) concluded that the species *Vorticlava* (Alder) and *Acharadria* (Wright) (see Hincks, pp. 131–4) are immature forms of *Tubularia larynx*.

A search through the literature has shown that many authors, including Stechow (1925*a*), Pérez (1925) and Vervoort (1946), have mentioned the instability of the 'specific' characters of *T. larynx*. Sometimes new species are described which are almost certainly *T. larynx*, such as *T. sphaerogona* Hargitt 1927, *T. radiata* Uchida 1936, and *T. venusta* Yamada 1950. Stechow (1925*b*) describes *T. australis* as synonymous with *T. gracilis* Harvey and *T. gracilis* Lendenfeld, but Fenchel considered both these two species to be synonymous with *larynx*. The descriptions given by other authors are often insufficient to distinguish the material from *T. crocea* or immature *T. indivisa*.

The aim in this paper is to illustrate the variations that occur in the specific characters of *T. larynx* (based on Allman's description) and, where possible, to explain them.

The work was undertaken in Plymouth in 1952. I am grateful for the advice and help I received from the Director and Staff of the Laboratory. I wish to thank Dr W. J. Rees for the interest he has shown. I am further indebted to the Chairman and the Members of the R.O.S.C.M. Ltd. for permission to publish this work.

METHOD

The material examined was collected from buoys in the Sound or taken from a raft moored near the Breakwater. On being brought into the laboratory, the clumps were washed to remove the tubes of *Jassa falcata*. They were placed in glass bowls and the water was changed 3 or 4 times a day.

All attempts at culturing were unsuccessful.

The measurements of the heights of the stems are approximate—there is no morphological difference between the stem and the stolon. Measurements and counts were made with the aid of a dissecting microscope.

A variety of terms is used in describing Coelenterata. As far as possible, the terms used by Allman are retained. A tubularian colony is formed by several hydranths with a common coelenteron. A clump or cluster of hydranths is made up of several colonies often too densely entangled to permit separation. To standardize, the hydranths are described as mature or immature. An individual is considered mature when the gonophores contain spermatozoa or developing ova.

SPECIFIC CHARACTERS

Allman's description of *T. larynx* can be summarized as follows.

Trophosome

Hydrocaulus, numerous branching stems with more or less distinctly marked transverse annulations, 3·5–5·0 cm or more in length.

Coenosarc forming a collar-like expansion below the hydranth. In describing *T. polycarpa* Allman states that it has a fluted collar, as found in *T. larynx*.

Hydranth, 14–20 distal (oral) tentacles in two closely approximate alternate series, with about 20 proximal (aboral) tentacles each about 1 cm long. The hydranth is 0·5 cm wide across the widest part of the body.

Gonosome

Gonophores in long, pendulous clusters; in the male long simple racemes, longer than the aboral tentacles. In the female they are shorter and the peduncle (blastotyle) is branched to form a panicle or compound raceme. The male gonophore is elongated, the female globular oval in form.

Apical processes, four conical ones, larger in the female than in the male.

Radiating canals: no gastro-vascular canals.

Actinula

No oral tentacles at the time of liberation.

OBSERVATIONS

As will be shown, few of these characters are stable. Many of them have been found to vary according to the size of the hydranth, and this depends on age and stage of development.

For recognition purposes it has been found necessary to concentrate on 'mature' characters, ignoring 'immature'. Though *Tubularia larynx* forms a colony of individual hydranths with a common coelenteron, these are never all the same age. The age differences are accentuated by the phenomena of autotomy and regeneration. In most colonies, however, some of the hydranths will be mature.

Trophosome

Hydrocaulus. Branching has been found to be of universal occurrence and is frequent during early stages of colony formation when the stolon of the newly settled actinula grows along the substrate, forming the hydrorhiza. False branches are formed by actinulae growing on the parent stems. These are very prevalent in some colonies and the false are difficult to distinguish from true branches.

The length of the stem is very variable and has no bearing on the maturity of the hydranth, because of autotomy and regeneration. Colonies have been examined with stems varying from 1·2 to 17·0 cm in length. Environmental factors such as salinity and current force may affect growth in length. D'Arcy Thompson (1942) mentions experiments by Loeb (1906), which showed that stems regenerating in hypotonic sea water were larger than those in hypertonic or normal; the maximum increase was in 2·2% sea water. It was noticed that the longest colonies were taken from a raft which swung in response to the tidal currents: these colonies were unable to support the hydranths when placed in a jar, so weak were the stems. The weakness may be due to a thin perisarc secreted during a period of a fast growth brought about by factors other than current.

The factors affecting growth in length will also influence the occurrence of annulations in the perisarc. Berrill (1952*b*) has suggested that these represent surges in growth in *T. crocea* similar to those in other gymnoblastic hydroids described by him (Berrill, 1949*a*, *b*; 1952*a*) and by Hauschka (1944).

However, the rhythmic growth pulsations they record have never been seen in *T. larynx*, so this is not an adequate explanation for this species.

Growth in *T. larynx* occurs both at the stolon tip and in the stem below the hydranth, and it is in these areas that the annulations are formed. These areas are linked by the hydroplasmic tension in the coelenteron. Hydroplasmic pressure is defined by Hauschka (1944) as 'pressure over and above internal resistance of static form to compression from without'. The existence of such a pressure can be shown by cutting the stem below the growth region. The diameter of the growth region will be immediately reduced, showing that it has been under a pressure from within and that the thin perisarc is still flexible and unable to withstand compression from without; the stolon apex is similar in this respect.

The annulations are formed in these zones whilst the chitin is still malleable. Observations suggest that they are formed mechanically through the agency of the hydroplasmic tension. This force is stable throughout the length of the coelenteron, so an increase of pressure at any point will be transmitted throughout the coelenteron. Pressure is increased by the nutritive movements of the hydranth.

These movements appear co-ordinated and have been analysed as three cycles. First, the aboral tentacles are raised vertically, the hypostome contracts and its base swells, dilating the peristome and manubria of the gonophores. This circulates the fluids within the hydranth; the manubria expand and contract once or twice independently. The hypostome relaxes and lengthens, peristaltic waves move up and down its length, circulating nutritive fluids. Finally, the whole hydranth is pulled down on the stem by contraction of the growing region, apparently by longitudinal muscles. This last movement is occasionally preceded by the opening of the gland dividing the stomach from the coelenteron, resulting in the exchange of fluids between them. The movement itself will result in increased hydroplasmic pressure at the stolon, distending it. This could be called a 'growth surge', but it is not produced by regular pulsations of the coenosarc as are the pulsations described by Berrill and Hauschka. Furthermore, when there are several hydranths with a common coelenteron their movements being unco-ordinated will produce varying pressures at the stolon apex. After the autotomy of a hydranth of a colony the hydroplasmic pressure induced by the others will be applied to the area of regeneration, possibly resulting in a reduction of stolonic growth.

Each distension at the stolon apex is limited in circumference by the chitin secreted by the coenosarcal ectodermis. The hardening of the chitin posterior to the apex results in the formation of annulations, marking each successive distension or 'growth surge' of the apex. The annulations are less regular and are often incomplete in the growth region below the hydranth where the downward movement of the hydranth results in a concertina-like in-folding of the coenosarc (Fig. 1). A series of contractions will result in the formation of an annula by the hardening of the perisarc. Further annulae will be formed as growth takes place; their irregularity being due to variations in growth rate as well as to environmental factors which may influence the angle of growth. The length of the region of growth, where the perisarc is still pliable, will be limited mechanically by the ability of the coenosarc aided by hydroplasmic tension to support the hydranth erect—these are constant factors—as well as by any environmental factors which aid or hinder growth. The inter-relationship of these influences will be secondarily reflected in the frequency and size of the annulae. A slow-growing colony might then be expected to have a thickened well-annulated perisarc, whereas a fast-growing one would have a thinner and so less clearly annulated perisarc.

No colony examined during the period of this study has been entirely devoid of annulations.

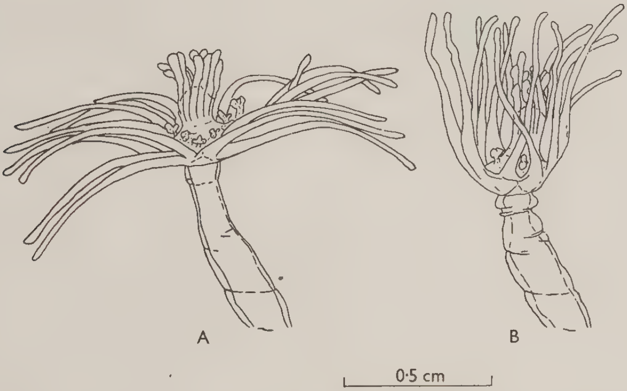

Fig. 1. A, immature hydranth with aboral tentacles extended horizontally. B, same, on completion of the third cycle of the nutritive movements—aboral tentacles vertical, peristome dilated and the growth region contracted folding the perisarc into annulae. (Drawn by camera lucida.)

Coenosarc

The coenosarc is said by Allman to form a collar below the hydranth similar to *T. polycarpa*.

This collar expands and contracts independently of the nutritive movements described previously. Beutler (1926) described these contractions as connected with the circulation of nutritive fluids within the coelenteron. Her diagrams are reproduced in Borradaile, Potts, Eastham & Saunders (1948). These contractions have been seen more frequently in the mature ageing polyp which has ceased to grow in length; the perisarc is stiff right to the base of the hydranth which is thus restricted in its movements. Such individuals are thought to be ageing because the clusters of gonophores on the peristome appear to prevent the aboral tentacles from rising to the vertical. The collar itself increases in circumference with the growth of the hydranth and there is little doubt that this increase is reflected in the increasing circumference of the perisarc of the stem, as compared with that of the hydrorhizal area.

It is difficult to decide whether this collar is itself a specialized organ or merely a dilation differentiated mechanically by the nutritive movements and the stomach contractions. The latter is the more probable explanation. It is recognized first some days after the settled actinula has started to feed and it increases in conformity with the width of the hydranth.

The fluting is formed by chitin secreted by the ectoderm and furrowed by the muscle movements of the collar.

Hydrorhiza

Allman gives no specific characters for the hydrorhiza. It is difficult to differentiate stem from hydrorhiza except where the latter is attached to the substrate. The hydrorhiza is typically of smaller diameter than the stem.

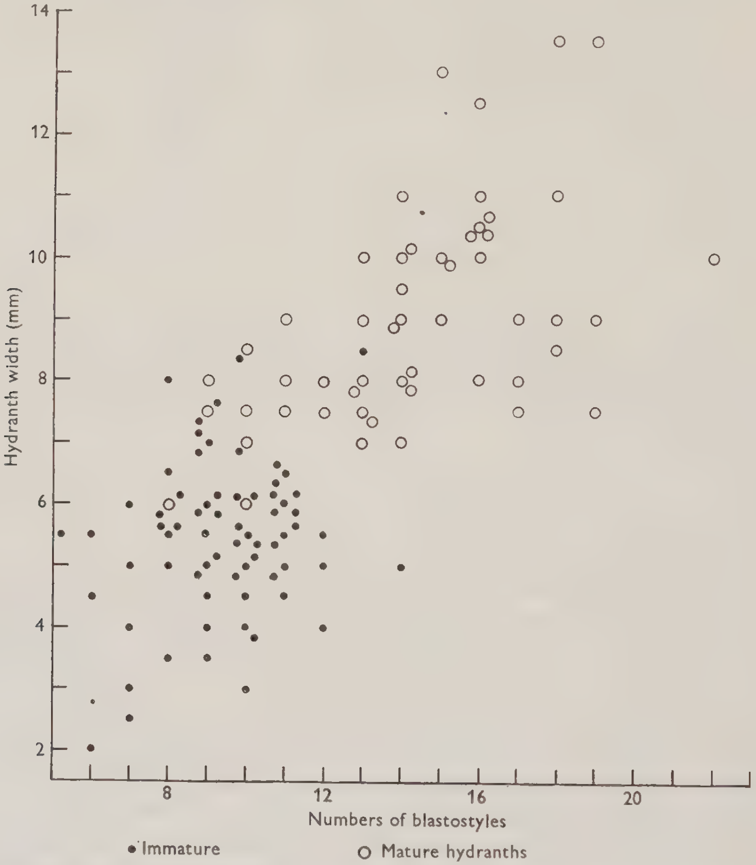

Fig. 2. Scatter diagram illustrating the correlation between the numbers of blastostyles and the widths of the hydranths. (Data in Appendix A.)

Hydranth

In describing the hydranth Allman describes his species as being of definite widths or having definite numbers of tentacles. The reliance given by other workers to these figures has resulted in the erection of invalid species.

Examination of many clusters showed these characters to be very variable.

A clump of 120 hydranths was then examined in detail. All individuals with gonophores, whether mature or not, were examined and the numbers of tentacles and gonophores, the sex and the widths of the hydranths were recorded (Appendix A). There is an intercorrelation between width, numbers of blastotyles and numbers of aboral tentacles. Figs. 2 and 3 show this, demonstrating that an increase in hydranth size is accompanied by increases in numbers of tentacles and blastostyles, which is to be expected. Any identification based on such characters must allow for this correlated increase.

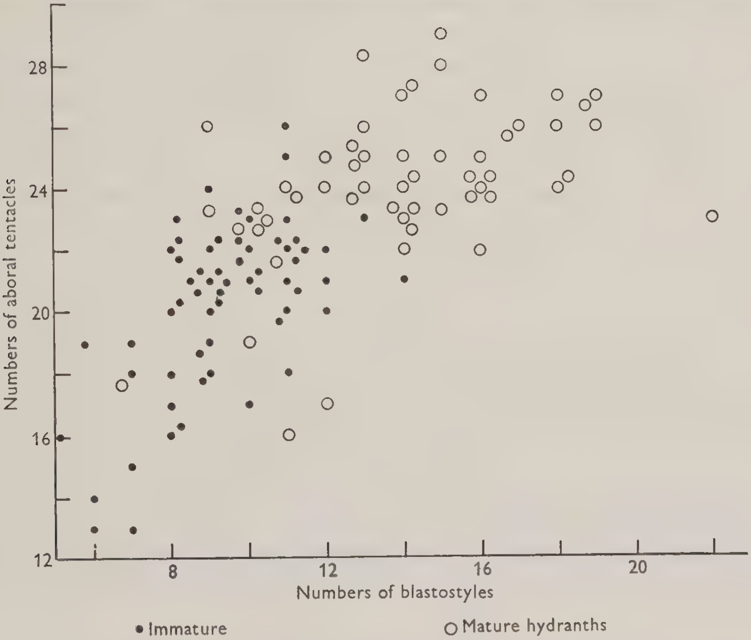

Fig. 3. Scatter diagram showing the correlation between the numbers of blastostyles and of aboral tentacles. (Data in Appendix A.)

Gonosome

Gonophores. In general, the mature male gonophores are smaller than the mature female; they are oval whereas the female are round. Moreover, the developing ovum displaces the manubrium within the gonophore. A male hydranth appears whiter because of the dense white spermatozoa and spermatocytes within the gonophore, mature and immature being distinguished from each other by size. Immature males and females are frequently indistinguishable.

Hermaphrodites have been found. The apical gonophores of a mature and ageing female hydranth are frequently male.

Liu & Berrill (1948) and Berrill (1952*b*) have recorded hermaphrodites in *T. crocea*. Berrill also terms such colonies as ageing. He suggests that male gonophores are budded on a female blastostyle as the result of a reduction in growth capacity; a maximum growth rate being necessary for the initiation of the large female gonad. When this is not attained male gonophores develop in place of female.

In support of this suggestion is the observation made earlier that such individuals are considered aged because the nutritive movements of the hydranth are restricted by the numbers of blastostyles and gonophores on the peristome. The hardening of the perisarc below the hydranth reduces the exchange of nutritive fluids between the coelenteron and hydranth. This may cause a local reduction in growth capacity, resulting in the development of male gonophores on a female blastostyle, for the reasons suggested by Berrill.

Pérez (1939) states that at Wiméreux *T. larynx* is always hermaphrodite. He has probably mistaken immature gonophores on a mature female blastostyle for male.

Apical processes. These are fairly consistent characters. They are not of the same size on all the gonophores, those of the male are often smaller than the female. Variation occurs in the numbers of processes as well as in their length.

Actinula

The actinulae of *T. larynx* are described by Allman as not having oral tentacles at the time of liberation. Apparently, the development of the oral tentacles can occur before or after release from the gonophore. At the same time it has been noticed that when the tentacles are not formed the hypostome is rounded and on it can be seen dark areas of tissue approximating to the future positions of the tentacles. The hypostome elongates at the same time as development of the tentacles. On occasions development has been delayed until after settlement.

Material Collected in Plymouth Sound

The material to be described can be divided into that collected from a raft near the Breakwater, over a period of several weeks, and that collected from various buoys, etc., in other parts of the Sound. Mature specimens were found at all times between June and late September, though they became scarce in August, when clumps without hydranths were frequently found.

Trophosome

Hydrocaulus. Length 1·5–17·5 cm. It was noticed that the perisarc of the longer stems—10 cm and more—was soft and thin in contrast with that of smaller individuals. The longest stems were those of a clump growing attached to a piece of twine trailing from the raft. In general, the material from the raft was of a greater length than elsewhere.

Regular annulations were common and more pronounced in the shorter stems, in which branching was more frequent. It is suggested that in the longer colonies collected from the raft where the current was persistent the actinulae were released and swept away from the parent hydranth. In colonies where the current direction varied, some actinulae are trapped amongst the parent stems to which they become attached, increasing the amount of apparent or false branching.

Ceonosarc. A collar occurs beneath the hydranth and it increases in size with age. It appears to be fluted in ageing individuals for reasons given earlier.

Hydrorhiza. Branching and annulations are frequent. This zone is smaller in circumference than the stem.

Hydranth. The numbers of tentacles show great variation; an increase in circumference of the oral cone and the peristome is accompanied by an increase in the numbers of tentacles. Examples of the amount of variation that occurs in mature individuals is recorded in Appendix B.

Gonosome

Gonophores. Blastostyles occur in one or two rows on the peristome; the number of rows increases according to the size of the hydranth. In the sample (Appendix A) the blastostyles vary from 5 to 22. The gonophores are occasionally pendulous; up to 35 have been counted on a single blastostyle.

Apical processes. Usually four in both sexes—occasionally less—easily distinguished in the female but seldom of equal size.

Actinula

Oral tentacles are usually developed prior to liberation from the gonophore. Numbers of aboral tentacles have varied between 7 and 11, and oral between 3 and 5.

DISCUSSION

Brink (1925*a*) remarked that '...the weak point of hydroid systematics is due to the fact that the study of the variability of external features has not kept pace with the discovery of new species'. He drew attention to Fenchel's (1905) examination of the literature, which demonstrated this point. Many new species have been described since.

There is no doubt that the material described here constitutes a single species. Sufficient variation can be found within a large clump to cover many of the species described by Allman. For instance, *T. bellis* differs from *larynx* only in the height of the colony described. Similarly, in *T. humilis* he describes the 'male'; the small number of gonophores suggests that Allman was describing an immature, recently regenerated hydranth. A similar error could have been made in the species *T. attenuata*. *T. polycarpa* owes its creation to having been found on the bottom of a ship in Coquimbo harbour.

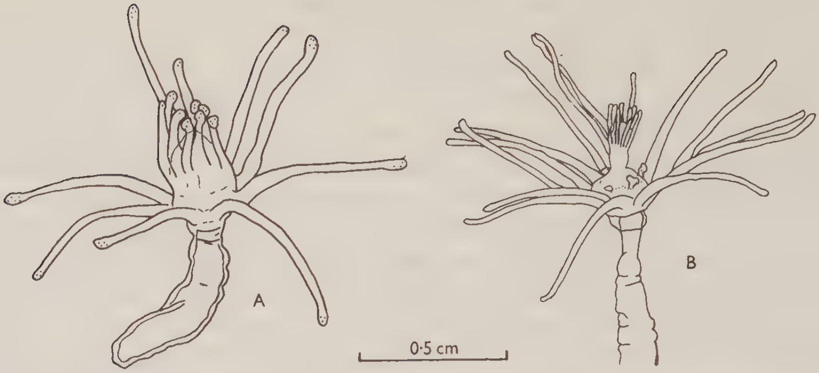

Fig. 4. A, a recently settled actinula drawn by camera lucida. Compare with Hincks, *Vorticlava*, Vol. 1, p. 132, and Vol. 2, pl. XXIII. B, an immature regenerated hydranth. Compare with Hincks, Vol. 2, pl. XXIII. (Drawn by camera lucida.)

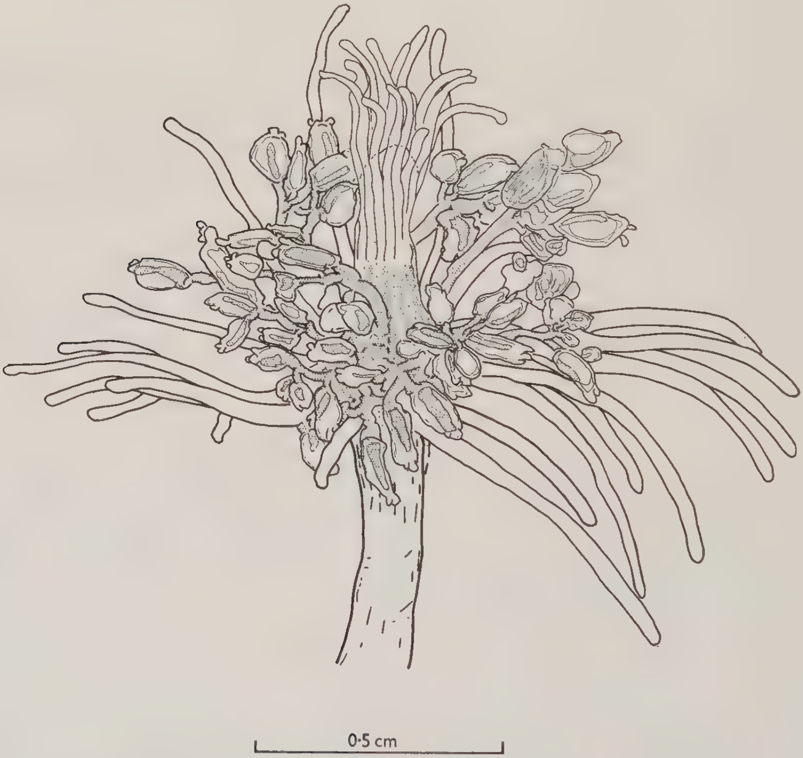

Fig. 5. Mature female hydranth showing the displacement of the manubria, within the gonophores, by the developing ova and actinula. (Drawn by camera lucida.)

Brink (1925*b*) considered the species of *Vorticlava* and *Acharadria* to be stages in the development of *T. larynx*. From Alder's description *Vorticlava* is certainly a newly settled actinula, as suspected by Hincks. Wright's drawing of *Acharadria*, however, resembles more closely an immature, regenerating hydranth, rather than a stage in the growth of a colony, because of the large number of tentacles and simultaneous absence of gonophores.

It is not possible to describe accurately the geographic distribution of *T. larynx*. It is certainly found in all oceans but it is, as yet, recorded under many different specific names.

It must be emphasized that Allman's specific characters are valid when full allowance is made for the diversity that occurs in the species. This is reduced when several mature individuals are used in identification.

SUMMARY

As certain previous authors have observed, *T. larynx* is a species subject to much variation. Disregard of this has led to the erection of invalid species.

The descriptive characters used by Allman are discussed. In a comparison with material collected in Plymouth Sound these characters are shown to be so variable that used singly they are valueless. In particular, disregard of the stage of development and over-emphasis on size will be very misleading.

The causes of some of this variability are suggested.

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APPENDIX A

DATA OBTAINED FROM EXAMINING A CLUMP OF *TUBULARIA LARYNX* COLLECTED ON A BUOY NEAR REMAINS OF THE PIER

(The graphs are drawn from these data. M = mature; I = immature.)

Sex		Hydranth width (mm)	Length aboral tentacles (mm)	Nos. tentacles		Blastostyles	
				Oral	Aboral	Nos.	Rows
♀	M	7·5	—	22	27	19	2
♀	M	10·5	—	20	24	16	2
♀	M	10·0	—	21	27	16	2
♂	M	8·0	—	17	22	14	1
♀	M	10·0	—	21	26	13	2
♀	M	10·0	—	22	23	22	2
♀	M	10·0	—	20	29	15	2
♀	M	11·0	—	22	24	18	2
♀	M	12·5	—	23	24	16	2
♀	M	8·0	—	19	28	13	2
♀	M	10·5	—	22	24	16	2
♀	M	9·0	—	20	26	19	2
♀	M	7·0	—	16	23	10	1
	M	6·0	—	18	22	8	1

Appendix A (*continued*)

Sex		Hydranth width (mm)	Length aboral tentacles (mm.)	Nos. tentacles		Blastostyles	
				Oral	Aboral	Nos.	Rows
♀	M	7·5	—	21	26	17	1
♀	M	8·0	—	20	26	9	1
♀	M	8·5	—	23	26	18	2
♀	M	8·0	—	21	24	12	1
♀	M	8·5	—	20	23	10	1
♀	M	11·0	—	20	24	16	2
♀	M	13·0	5·0	19	25	15	2
♀	M	8·0	3·0	20	17	12	1
♀	M	11·0	—	23	25	14	2
♀	M	9·5	3·0	18	27	14	2
♀	M	8·0	—	22	27	14	2
♀	M	8·0	3·5	21	25	13	2
♀	M	7·5	3·0	16	23	9	1
♀	M	7·5	2·5	19	25	12	1
♀	M	9·0	—	19	27	18	2
♀	M	7·0	3·0	19	21	9	1
♀	M	10·0	3·7	23	23	14	2
♂	M	6·0	2·5	18	23	10	1
♀	M	10·0	3·7	19	23	14	2
♀	M	8·0	3·0	21	22	16	2
♀	M	9·0	3·0	18	22	11	1
♀	M	13·5	5·0	24	24	18	2
♀	M	6·0	2·0	19	22	9	1
♀	M	8·0	3·5	22	24	11	2
♀	M	13·5	5·0	19	27	19	2
♀	M	10·0	3·5	23	28	15	1
♀	M	9·0	3·0	24	25	13	—
♀	M	9·0	3·5	18	23	14	2
♀	M	10·5	3·7	21	25	16	2
♀	M	7·5	3·0	21	23	10	1
♀	M	9·0	3·5	25	26	17	2
♀	M	10·5	4·5	22	24	16	2
♀	M	9·0	3·5	22	24	14	1
♀	M	7·0	3·0	22	24	13	1
♀	M	7·5	3·0	19	25	13	1
♀	M	8·0	3·0	—	24	14	1
♀	M	7·0	3·0	23	23	14	1
♀	M	9·0	3·5	20	23	15	1
♀	M	5·0	2·0	12	16	11	1
♀	M	7·5	3·0	18	24	11	1
♀	I	5·5	—	20	20	12	1
♀	I	7·0	—	26	23	10	1
♀	I	8·5	3·0	23	23	13	1
♀	I	8·5	3·5	18	22	10	1
♀	I	6·0	—	14	22	11	1
♀	I	7·5	2·5	18	24	9	1
♂	I	6·0	2·5	18	23	10	1
♂	I	6·0	2·5	20	22	11	1
♀	I	6·0	2·2	14	21	9	1
♀	I	7·5	3·2	16	21	9	1
—	I	7·0	2·5	18	21	9	1
—	I	6·0	2·0	19	22	9	1
—	I	6·0	2·5	22	18	7	1
—	I	8·0	3·5	19	23	8	1
—	I	4·0	1·5	19	21	10	1
—	I	7·0	3·0	15	19	9	1
—	I	5·5	2·2	19	22	10	1
—	I	5·5	2·0	15	16	5	1
—	I	2·5	0·75	—	15	7	1
—	I	2·0	0·75	16	13	6	1
—	I	6·0	2·5	17	16	8	1
—	I	3·0	1·2	14	17	10	1
—	I	5·0	—	20	21	10	1

APPENDIX A (*continued*)

Sex		Hydranth width (mm)	Length aboral tentacles (mm.)	Nos. tentacles		Blastostyles	
				Oral	Aboral	Nos.	Rows
—	I	5·0	—	19	21	11	1
—	I	5·0	—	16	21	9	1
—	I	5·5	—	19	20	11	1
—	I	3·5	—	10	16	8	1
—	I	5·0	—	20	22	12	1
♂	I	6·0	2·5	21	21	9	1
♂	I	6·0	2·5	17	22	10	1
♂	I	4·5	1·7	14	20	10	1
♀	I	6·5	2·7	21	22	11	1
♀	I	5·5	2·2	16	20	8	1
♀	I	5·5	2·5	18	14	6	1
♀	I	4·0	1·5	18	19	7	1
♀	I	5·5	2·2	19	20	10	1
♀	I	6·5	2·7	16	22	8	1
♀	I	5·5	1·5	15	20	11	1
—	I	5·0	2·0	22	19	10	1
—	I	5·5	2·2	15	21	10	1
—	I	5·0	2·0	18	22	8	1
—	I	6·0	2·5	17	22	9	1
—	I	5·5	2·2	18	22	11	1
—	I	3·5	1·2	16	18	9	1
—	I	5·5	2·2	19	20	8	1
—	I	5·5	2·2	22	19	10	1
—	I	6·0	2·5	15	22	11	1
—	I	4·0	1·5	14	18	10	1
—	I	6·0	2·5	18	21	11	1
—	I	4·0	1·5	17	21	12	1
—	I	6·5	2·7	20	23	11	1
—	I	5·5	2·2	21	17	8	1
—	I	5·0	2·0	19	21	14	—
—	I	6·0	2·5	19	26	11	—
—	I	4·0	1·5	15	19	9	1
—	I	5·0	2·0	18	19	10	1
—	I	5·0	2·0	18	18	9	1
—	I	6·0	2·2	16	18	8	1
—	I	4·5	1·7	17	21	9	1
—	I	6·5	2·7	21	25	11	1
—	I	4·5	1·7	14	19	6	1
—	I	4·5	1·7	17	18	11	1
—	I	3·0	1·2	15	13	7	1
—	I	5·0	2·0	14	18	7	1
—	I	5·0	—	20	21	10	1
—	I	5·0	—	19	21	11	1

APPENDIX B

RECORDS OF CLUMP HEIGHTS AND TENTACLE NUMBERS OF MATURE INDIVIDUALS IN CLUMPS COLLECTED IN VARIOUS PARTS OF PLYMOUTH SOUND

Locality	Clump height (cm.)	Variation in tentacle nos.		No. examined
		Oral	Aboral	
Buoy chain, breakwater	1·5–4·0	17–26	18–25	12
Buoy chain, Barn pool	1·5–5·0	14–37	13–35	29
Buoy chain, Mount Batten	1·5–5·0	17–22	18–25	30
Raft, breakwater	1·5–5·0	16–22	20–26	90
Raft, breakwater	5·0–7·5	21–31	22–29	
Raft, breakwater	7·5–12·5	17–32	19–32	
Pier, buoy	1·5–7·0	12–24	16–28	54
Total range of variation	1·5–12·5	12–37	13–35	215

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THE DIET AND FEEDING MECHANISM OF *IDOTEA*

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(Text-figs. 1 and 2)

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INTRODUCTION

Few published data are available on the feeding mechanism of isopods; Nicholls (1931) describes the feeding of *Ligia oceanica* (L.), but he deals mainly with the foregut and the digestive system. The feeding mechanism of the tanaid *Apseudes talpa* (Montagu) is described by Dennell (1937) but, unlike an isopod, this species still retains a fairly elaborate maxillary filter mechanism. A description of the mode of action of the mouthparts of an isopod such as *Idotea* is therefore of value for comparison with the general scheme of evolution of peracaridan-feeding mechanisms (Dennell, 1937). In addition, such an account forms a corollary to morphological descriptions of the mouthparts (Naylor, 1955*a*), and notes on the diet of *Idotea* supplement observations on the ecology of the various species (Naylor, 1955*b*).

DIET

According to Roux (1829) and Collinge (1917), idoteids feed mainly on animal remains, but Bate & Westwood (1868) maintained that *Idotea tricuspidata* (now *I. baltica* (Pallas) and other species) fed on algae. As for the habits of individual species, Dollfus (1895) states that *I. pelagica* Leach is commensal with, or parasitic on, barnacles on the shore, Kjennerud (1952) describes how *I. neglecta* G. O. Sars eats fish and fish waste in the fish-market harbour at Bergen, and Howes (1939) regards *I. viridis* (Slabber) from a saline lagoon in Essex as a carnivore feeding chiefly on coelenterates, *Membranipora* or dead organisms.

Present observations on the feeding habits of *Idotea*, when compared with details of the ecological distribution of the species (Naylor, 1955*b*), show how each species eats the organisms with which it is associated. Examination of the gut contents shows that most of the food is algal, but some animal remains have been observed, particularly in *I. pelagica*, which often has barnacle appendages in its gut. In addition, laboratory observations show that an idoteid will eat its own cast skin and other dead *Idotea*; cannibalism, particularly of moulting individuals, sometimes occurs.

To determine whether plant or animal food is preferred *Idotea* were kept in aquaria and fed experimentally. *I. emarginata* (Fabricius) and *I. neglecta* ate *Laminaria* and scallop muscle if both were provided as food, and *I. emarginata* attacked and ate living *Arenicola*, whether seaweed was present or not. Dead fish such as whiting, herring, pollack and coalfish were eaten till nothing but the skeleton remained, even when *Laminaria* had been the food for several days.

Thus, though seaweeds seem to provide the bulk of available food for these species in their particular habitat, animal remains, and even some living animals, are taken when available.

Probably most *Idotea* are omnivorous, but some feeding differences occur depending on the habitat and locality. *I. pelagica* appears to have similar habits in many localities (Dollfus, 1894–5; Sars, 1899; Elmhirst, 1946; Kjennerud, 1952; Naylor, 1955*b*). The species is found amongst barnacles on exposed shores, but rather than being an ectoparasite of barnacles (Dollfus, 1894–5), it seems as though it is a scavenger in its habitat, feeding on cast skins of previously moulted barnacles, as well as on algae. Local differences in diet occur in at least two species. *I. neglecta* appears to feed largely on decaying weed in Port Erin Bay, yet the species appears to be solely carnivorous in a Norwegian locality (Kjennerud, 1952), and *I. viridis* in the Isle of Man feeds chiefly on algae and not on animal matter as described by Howes (1939). Finally there may also be different food preferences by *Idotea* of different size; small *I. granulosa* Rathke, for instance, eat the *Cladophora* with which they are associated, whilst large specimens eat the fucoids which harbour them (Naylor, 1955*b*).

The Feeding Mechanism

Methods

In favourable conditions, without excess light or heat, *Idotea* will feed on algae in a Petri dish, and may be examined under a low-power dissecting microscope. In this way, part of the feeding process, particularly the biting and scraping of the weed surface, was observed directly. The remainder of the process of feeding is inferred from the structure and musculature of the mouthparts, and by observing the movements of the mouthparts when the ventral maxillipedes, which cover the other mouthparts, had been removed.

The removal of the maxillipedes might upset the phase of movement of the mouthparts, but it did not seem to affect the direction of movement of each appendage.

The structure of the mouthparts

The structure of the mouthparts of *Idotea* has been described in detail elsewhere (Naylor, 1955*a*), but it is necessary to give a brief summary of that description here so that the mode of action of the mouthparts may be fully understood.

Idoteids have four pairs of oral appendages, namely, mandibles, maxillules, maxillae and maxillipedes, the last of these originating from the thoracic segment incorporated into the cephalon. In addition to these there are the labrum, forming the anterior wall of the oral cavity, and the bilobed paragnath, forming the posterior wall. The mandibles themselves form the lateral walls of the oral cavity. All the paired appendages originate behind the paragnath; they project forwards beneath the head with their tips coming to lie below or just behind the oral cavity (Fig. 1).

The *labrum* is a heavily chitinized structure which is slightly spiny at its lower edge (Fig. 1); it abuts against the food mass during feeding.

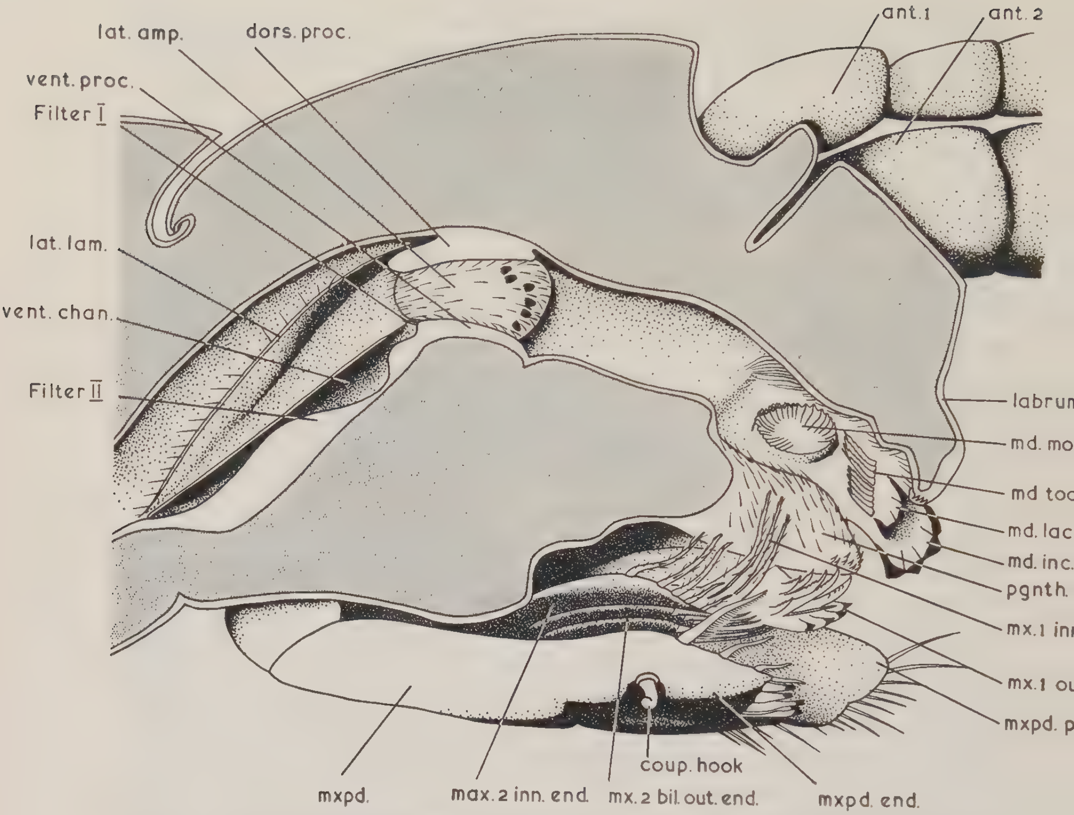
The *mandibles* are well developed and are the largest of the oral appendages. Each consists of a ventral incisor process, a lacinia mobilis, a row of toothed spines, and a dorsal molar process (Fig. 1), and when the mandibles are closely apposed the structures of the right appendage lie above the corresponding ones of the left side (Fig. 2). In relation to this the two mandibles are structurally asymmetrical; whereas the left lacinia mobilis bears three broad teeth, that of the right mandible has one large spine and several smaller ones and all are less chitinized than those of the left lacinia mobilis. In addition, the molar process of the left mandible is inclined upwards, whilst that of the right side faces somewhat downwards.

The *paragnath* is bilobed and bears upwardly-directed spines in the mid-ventral groove between the lobes.

Each *maxillule* has an inner endite bearing three long, and one short, plumose bristles which project between the lobes of the paragnath (Fig. 1), and an outer endite bearing a number of stout spines. Each *maxilla* has a bilobed outer endite bearing comb setae, and an inner endite bearing plumose bristles like those of the maxillule.

The *maxillipedes* are plate-like and they protect the other mouthparts. They are joined medially by a coupling hook (Fig. 1) on each appendage and, unlike the other appendages, the maxillipedes bear palps. The palps are capable of independent lateral movement, and the spines on their inner borders aid in keeping the other mouthparts free of debris.

The *first pair of legs* on the thorax aid in feeding by holding the food mass and by combing the mouthparts. Unlike the other legs, the dactylopodite is palmate and bears a number of toothed, comb-like spines on its upper surface.


lat. amp.
dors. proc.
ant. 1
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labrum
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pgnth.
mx. 1 inn
mx. 1 ou
mxpd. p
coup. hook
mxpd.
max. 2 inn. end.
mx. 2 bil. out. end.
mxpd. end.

Fig. 1. Median view of the left half of the head of *I. emarginata*, showing the mouthparts and the foregut. *Ant.* 1, first antenna; *ant.* 2, second antenna; *coup. hook*, coupling hook on maxillipede; *dors. proc.*, dorsal process; *lat. amp.*, lateral ampulla; *lat. lam.*, lateral lamella; *md. inc. proc.*, incisor process of mandible; *md. lac. mob.*, lacinia mobilis of mandible; *md. mol. proc.*, molar process of mandible; *md. tooth. sp.*, toothed spines of mandible; *mx. 1 inn. end.*, inner endite of maxillule; *mx. 1 out. end.*, outer endite of maxillule; *mx. 2 bil. out. end.*, bilobed outer endite of maxilla; *mx. 2 inn. end.*, inner endite of maxilla; *mxpd.*, base of maxillipede; *mxpd. end.*, endite of maxillipede; *mxpd. palp*, palp of maxillipede; *pgnth.*, paragnath; *vent. chan.*, ventral channel; *vent. proc.*, ventral process.

The Mouthparts in action

General Movements

During feeding the mandibles bite sideways. The maxillules move obliquely forwards till they meet in the mid-line and then they may or may not move forwards together. It is important to note that the mandibles and maxillules alternate in their movements.

The maxillae move in a manner similar to that of the maxillules, converging anteriorly to about the same level as the posterior limit of the tips of the maxillules. The maxillipedes, being coupled together medially, move forwards and backwards, with no lateral movement except in the palps.

All these movements may vary according to whether the animal is actually biting the food material or only clearing the mouthparts of debris collected there.

Biting and Scraping

From a functional point of view all the mouthparts but the maxillae are built on a similar plan. Ventrally placed on each appendage are heavily chitinized structures for biting or scraping the food material. There are broad, scleratized teeth on the incisor process of each mandible, and on the lacinia mobilis of the left appendage. The outer endite of the maxillule has four, heavily chitinized, chisel-shaped spines ventral to the other spines, and the maxillipedes have five or six scraping spines situated ventrally on their inner parts.

When the animal is feeding, the maxillules come together as the mandibles separate, and in doing so they abrade the edge of the weed and facilitate the work of the mandibles when these next bite. To a certain extent the spines on the maxillipedes probably also help in abrading the weed, but this pair of appendages, like the maxillules, do not bite the food material.

The maxillae take no part in abrading or biting the weed; they are hardly scleratized at all.

Pushing

Above the biting and scraping parts of the appendages are structures concerned with the removal of food from the biting parts and with its transfer to the molar processes for chewing. Serving to transfer food upwards along the mandibles are the row of toothed spines and the lacinia mobilis of each side (Fig. 2). By their very arrangement these should push food upwards every time the mandibles come together. Cannon & Manton (1927, p. 235), in describing the asymmetrical arrangement of the mandibles in *Hemimysis*, state that it provides 'a mechanism by which simple lateral movements of the mandibles must transfer food from the ventral incisor processes to the dorsal molar processes'.

In addition to those on the mandibles there are toothed spines on the maxillules. These lie dorsal to the four scraping spines (Fig. 1) and they point upwards and inwards between the paragnaths; they must aid in pushing the bitten food mass upwards. Here again it is important that the mandibles and maxillules alternate in their movements. At the time when the food bitten by the mandibles is likely to fall between them as they retract, the maxillules are coming together; in doing so they will push inwards any food which is likely to fall, and the toothed nature of the spines should make them more efficient in this respect.

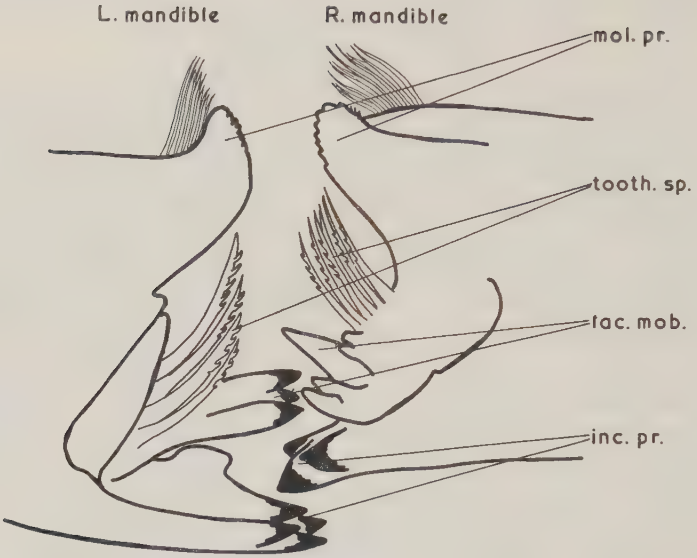

Fig. 2. The apposed mandibles, seen from behind (*mol.pr.*, molar process; *tooth.sp.*, toothed spines; *lac.mob.*, lacinia mobilis; *inc.pr.*, incisor process).

Brushing

Escaping particles of food are probably dealt with by the plumose, or brush setae, on the maxillules, maxillae and maxillipedes. It is difficult to observe these setae in action but, from the general movement of the appendages, some idea of their function may be inferred.

The plumose setae move mainly forwards and backwards, since they are on the inner parts of the appendages, and the setae of each member of the pair of appendages meet in the mid-ventral line. The three pairs of appendages normally move out of phase with each other, and particles of food are probably brushed forwards from one to the other until they are combed off the setae of the maxillules by the spines on the paragnath.

From there the food particles will be pushed between the mandibles by the toothed spines on the outer endite of the maxillule, and by the brush setae on the inner endite of that appendage. Once between the mandibles, as described earlier, food passes up to the molar processes by virtue of lateral movements of the mandibles themselves.

Combing

It is necessary in such a method of feeding, where small particles of food will tend to cling to various appendages, for there to be combing mechanisms to remove such debris. Comb spines on the first pair of legs and the long smooth spines on the inner border of the maxillipedal palp, have already been described as aiding in keeping the mouthparts free of debris. In addition, short smooth spines on the paragnath have been mentioned as combing food particles from the brush setae of the inner endites of the last three pairs of mouthparts. Other structures which probably aid in combing the mouthparts are the spines on the outer endites of the maxillae (Fig. 1), and the two flexible, blade-like structures, fringed with stiff bristles, which are situated ventrally on the tips of the maxillules (Naylor, 1955*a*).

Trituration

All the movements of the mouthparts serve to push the food forwards and upwards to the molar processes of the mandibles. These have blade-like ridges on their apposing surfaces, which slide across each other to crush the food. From there the triturated food passes to the fore-gut.

The Foregut

The foregut of *Idotea* is very similar to that described for *Asellus* (Rehorst, 1914) and for *Ligia* (Nicholls, 1931). The latter author reviews much literature on the foregut of isopods, and the terminology adopted by him is used here.

Anteriorly, on each side-wall of the foregut, arise the paired, lateral ampullae. These appear to crush the food material so that fluid is sifted from the solid matter through paired, ventral bristle-plates, which open into a mid-ventral channel (filter I). The channel is formed by the anterior ventral lamellae, and it is divided medially, at its posterior end, by a second filter apparatus (filter II). On either side of this structure, running parallel with its upper edge, lies a groove covered with strong bristles. The grooves are blind anteriorly, but posteriorly they open into the three digestive caeca of each side; fluid passes between the setae and into the caeca. A backward projection of the second filter apparatus acts as a valve over the opening from the caeca into the intestine.

Solid food is prevented from being regurgitated by a series of membranous flaps in the foregut, namely, the paired lateral lamellae, the dorsal lamella, the paired, posterior ventral lamellae, and the ventral valve. All except the lateral lamellae arise at the posterior end of the foregut and project into the intestine.

DISCUSSION

From what has been said of the diet and action of the mouthparts, it is clear that *Idotea* must feed on large food masses; it cannot filter food from suspension, and in this respect it is a typical 'higher' peracaridan (Dennell, 1937). The general method of feeding of *Idotea*, whereby the animal browses on its food, scraping and biting pieces from it, is similar to that described for *Ligia* (Nicholls, 1931).

The 'higher' Peracarida, which feed on large food masses, are thought to have been derived from an entirely filter-feeding ancestor (Cannon & Manton, 1927; Manton, 1928; Dennell, 1937), and the first adoption of the bottom-living habit by a peracaridan with an elaborate maxillary filter may have been the result of seeking larger food particles from the bottom (Manton, 1928). The first stage in obtaining larger food appears to have been the direct intake of fairly large particles by the mandibles, the outer endites of the maxillules, and possibly other appendages as well; this has been observed in *Hemimysis* (Cannon & Manton, 1927), and in *Apseudes* (Dennell, 1937). It is then an easy step towards the elaboration of the mandibles and the outer endites of the maxillules, so as to bite into, and break pieces from, more solid food.

As the filter-feeding mechanism has become of less importance in the 'higher' Peracarida, the inner endite of the maxillule, and the whole of the maxilla, have become reduced, and the mouthparts have become covered ventrally by the plate-like maxillipedes. All these changes appear to have taken place with the loss of the ventral food current, and they have occurred in the evolution of the Isopoda.

In *Idotea* the equivalent of the filtering mechanism, namely the inner parts of the hinder three pairs of mouthparts, apparently serves to brush into the mouth particles of food which escape from the biting parts of the appendages. The mouthparts are very compact, but the feeding mechanism is still elaborate, particularly when the division of labour between the various parts of each appendage is considered.

With the adoption of a bottom-living habit and a raptatory mode of feeding, characteristic of the 'higher' Peracarida, and with its generalized food requirements (p. 348), *Idotea* is well suited to occupy a wide range of habitat.

SUMMARY

Idotea feeds on large food masses. It is potentially an omnivorous scavenger, but each species may have a characteristic diet depending on the availability of food in its particular habitat.

The structure, topography and action of the mouthparts are described: spines on the maxillules and maxillipedes scrape the food material whilst the mandibles actually bite the food; toothed spines on the maxillules and mandibles push the food upwards to the molar processes of the mandibles; debris is brushed forwards between the lobes of the paragnath by setae on the inner endites of the maxillules, maxillae and maxillipedes; and spines on the maxillipedal palp, and on the first leg, comb the mouthparts.

The structure of the foregut resembles that of *Asellus* and *Ligia*.

The relation of the feeding mechanism to the functional evolution of the mouthparts of Peracarida as a whole is discussed.

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OBSERVATIONS *IN VIVO* ON THE BREEDING OF *ELMINIUS MODESTUS* GROWN ON GLASS SLIDES

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(Text-figs. 1–7)

Periodic sampling and examination of the gonads or retained embryos from a given species may fail to give reliable information on the number and frequency of its broods. When several broods follow one another in rapid succession and are not synchronous, as occurs at the height of the breeding season in some animals, the gonads are to be found in all stages between the immature and spent condition. If embryos are retained they also occur in all stages of development. The relative time occupied by any one of these stages, expressed as a fraction of the time taken by a single brood, is equal to the proportion of the population which occurs in that stage of development. This can readily be determined by appropriate sampling.

On the contrary the actual time occupied by a single brood (namely the time between the liberation of one brood and the next) cannot be obtained from such records. Only when continuous observations can be made on the breeding of separate individuals is it possible to determine directly the duration of each brood. Where the proportion of breeding individuals in a population increases only slowly, but the brood cycles are short, this may indeed afford the only satisfactory method.

It is well known that the state of the female gonad in operculate barnacles can be determined visually if the individual is removed from its substratum and viewed from below. Moore (1935) illustrates the difference between unfertilized and fertilized individuals of *Balanus balanoides*. Individuals carrying on each side of the mantle cavity recently extruded egg masses have a characteristic appearance both in the Balanidae and in the Veruccidae. Moreover, so far as is known, oviposition in these groups occurs only immediately after copulation. Unfortunately removal from the substratum is normally necessary for the purpose of such an examination and this precludes further observations on that individual. If, however, the species has a transparent basis, and can be grown on a transparent substratum, visual observations may be continued indefinitely. Moreover, since during development the egg masses become increasingly pigmented the darkening of colour provides a useful guide to the rate of development.

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Elminius modestus, having a transparent membranous basis and breeding most prolifically during the warmer months (Crisp & Chipperfield, 1948), was considered to be a very suitable organism for this work. Unlike *Chthamalus stellatus*, which also breeds prolifically over the greater part of the summer (Crisp, 1950), *Elminius* will settle readily on, and remain attached to, smooth glass surfaces.

Methods of Investigation

The observations described below were carried out in Brixham Harbour, South Devon, on individuals of *E. modestus* attached to glass plates of dimensions $4 \times 3 \times \frac{1}{4}$ in. Thinner glass plates improve visibility, but are liable to accidental damage during immersion in the sea. These plates fitted into bakelite frames, which were bolted to panel holders (Fig. 1 *a*), enabling them to be suspended from a raft at a depth of 6 ft. below the surface of the sea. It was found advisable to use the inner wells of the raft (Fig. 1 *b*) in order to reduce incident illumination, since strong light in clear water promoted dense algal growth, which smothers *Elminius*. As a further precaution against interference by other organisms, the barnacles were occasionally cleaned by gently scrubbing the surface of the plate with a nail-brush and washing with fresh water, to which *Elminius* is known to be extremely tolerant.

Although a small regular settlement of *E. modestus*, of the order of 0·01 per sq.cm during the season, is now (1952) maintained at Brixham, this was insufficient for our work. The initial settlements were therefore obtained by exposing plates in the River Crouch, Essex, where *Elminius* is exceedingly abundant, settlements of 50–100 spat per sq.cm having sometimes been obtained during only a week's exposure in June and July. The plates, each bearing an identification number, were taken to Brixham harbour (where all the observations were carried out), and immersed as soon as possible. When the spat had attained a diameter of 2–3 mm about fifty to sixty of the more healthy and larger individuals were selected, and the remainder prised off the panel, but no individual was allowed to be separated by a distance of more than 2 cm from its fellows. The panel was then photographed at intervals from the underside, the individuals in the photographs being numbered for reference.

When the spat reached maturity daily visits were paid to the raft whenever possible, in order to observe the condition of the female gonad of each individual. The newly fertilized egg masses can easily be picked out; by this means it became a simple matter to determine, to the nearest 1 or 2 days, the time at which eggs were extruded and hence when fertilization had occurred. Moreover, it is possible with practice to judge both the condition of the unfertilized ovary, from its colour and texture, and the approximate state of development of embryos, from the degree of darkening of the egg masses. These are at first a pale cream, then ochre, fawn and finally brown. A table was compiled in which was entered against the date and number of each barnacle, either a letter indicating the colour of the fertilized egg mass (e.g. W white, C cream, G grey, O ochre, F fawn, B brown), or E (empty), when no egg masses were present. The state of the ovaries was recorded by numbering 0, 1, 2 or 3, according to whether the ovaries were not discernible or were of small, moderate, or large size. A typical extract of such records is shown in Table I.

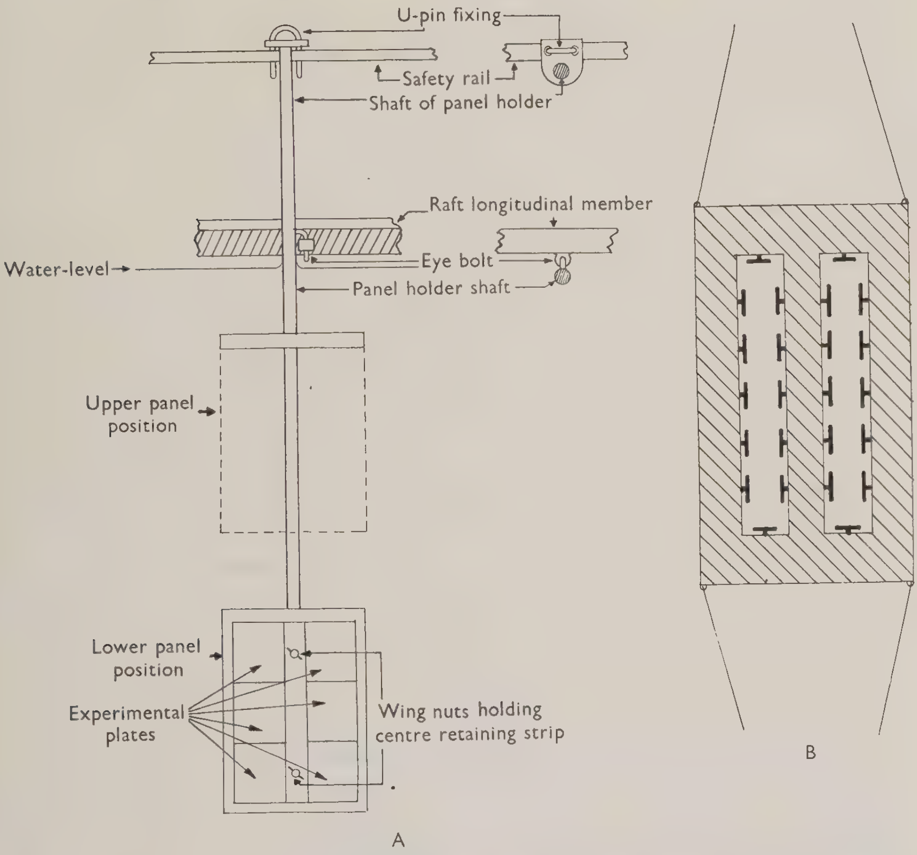
U-pin fixing
Safety rail
Shaft of panel holder
Raft longitudinal member
Water-level
Eye bolt
Panel holder shaft
Upper panel position
Lower panel position
Experimental plates
Wing nuts holding centre retaining strip
A
B

Fig. 1. Diagram of method of exposure of glass plates. A, Panel holder and fixing arrangements. B, Plan of raft showing wells for panel holders. The wells are darkened by wooden covers when the panel holders are in position.

In this table heavy lines are inserted to indicate either the liberation of nauplii or the fertilization and discharge of egg masses into the mantle cavity. It will be seen from the table that whenever a fertilization occurs, as shown by the sudden appearance of white or cream egg masses, the ovarian index figure drops sharply, usually from 3 to 0 or to 1 (e.g. individual 2, 22–25 April). This indicates the discharge of the greater part of the contents of the ovary, for the ovarian tubules at the time the individual is fertilized are almost entirely filled with mature eggs.

TABLE I. EXTRACT OF RECORDS OF BREEDING OF *ELMINIUS MODESTUS*

(Full lines represent fertilization or release. Figures indicate the development of the ovary, letters the state of fertilized eggs. W = white, C = cream, Y = yellow, G = grey, O = ochre, F = fawn, B = brown, E = empty, no fertilized eggs present.)

No. of individual		April																	May					
		4	5	6	12	13	14	15	17	19	20	21	22	25	26	27	28	29	2	3	5	8	10	12
1	Ovary	2	3	3	3	3	3	3	3	3	3	‖ 1	2	2	2	3	3	3	3	3	3	│ 3	│ 1	0
	Fert. eggs	O	?	E	B?	B?	B	E?	B	B	B	‖ C	O	B	B	B	B	B	B	B	B	│ E	│ C	G
2	Ovary	3	3	3	3	3	3	3	3	2	3	3	3	│ 0	1	1	1	1	2	2	3	3	3	3
	Fert. eggs	E	E	E	E	E	E	E	E	E	E	E	E	│ C	C	C	G	O	O	O	B	B	B	B
3	Ovary	2	│ 2	2	2	3	2	2	2	2	2	│ 0	0	2	2	2	‖ 1	2	3	2	3	3	‖ 0	0
	Fert. eggs	B	│ E	E	E	E	E	E	E	E	E	│ C	F	F	F	B	‖ G	B	B	B	B	B	‖ O	F
4	Ovary	2	3	3	2	2	2	2	2	2	2	3	2	3	3	│ 0	1	2	3	2	3	2	2	2
	Fert. eggs	E	E	E	E	E	E	E	E	E	E	E	E	E	E	│ C	G	F	F	F	B	B	B	B
5	Ovary	1	1	1	1	1	1	1	2	1	1	2	2	2	2	2	2	2	3	│ 0	1	1	2	│ 3
	Fert. eggs	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	│ C	C	O	F	│ E
6	Ovary	3	3	3	3	3	3	3	2	3	3	3	3	3	3	3	3	3	3	3	3	3	3	3
	Fert. eggs	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	(Later found infected)					
7	Ovary	1	1	1	2	3	3	3	2	│ 2	2	2	2	3	│ 0	0	1	1	1	2	2	3	│ 3	3
	Fert. eggs	G	G	G	F	B	B	B	B	│ E	E	E	E	E	│ W	C	C	G	G	G	G	F	│ E	E
8	Ovary	2	2	2	2	2	2	2	3	3	2	3	2	2	2	2	2	2	2	1	2	2	2	2
	Fert. eggs	E	E	E							(Infected with *Hemioniscus*)													
9	Ovary	2	2	2	2	3	3	3	3	│ 0	0	0	0	2	2	2	2	2	3	3	3	│ 3	│ 0	0
	Fert. eggs	E	E	E	E	E	E	E	E	│ C	G	G	O	F	F	F	F	F	B	B	B	│ E	│ C	C
10	Ovary	3	3	3	3	3	3	3	3	3	3	3	2	2	2	2	2	2	2	2	2	2	2	2
	Fert. eggs	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E	E
11	Ovary	1	2	2	3	3	3	3	3	‖ 0	1	1	1	2	2	2	2	2	3	3	3	‖ 0	1	1
	Fert. eggs	C	C	O	B	B	B	B	B	‖ C	C	C	C	G	Y	Y	Y	O	B	B	B	‖ C	C	C

It will also be noted that during the period of embryonic development as indicated by the changing colour of the fertilized eggs, the ovary gradually re-forms, there being a rise in the index of the ovary. In consequence a new batch of ova is often mature by the time the nauplii are being liberated, and another fertilization can immediately supervene, as in individual 1 between 20 and 21 April. When this takes place dark brown egg masses are replaced by new pale ones within the interval of time between successive observations, and double lines are inserted in the table. The embryos are normally retained for an appreciable period after reaching what appears to be a completely developed condition, for they are then fully pigmented, and hatch immediately if removed from the parent and placed in sea water.

The most difficult observation to make with certainty was the date of liberation. In healthy individuals which were feeding rapidly the growth of new ovarian tissue next to the basis sometimes obscured the darker colour and characteristic shape of the egg masses. This difficulty occurred mainly towards the end of development when the reconstituted ovary made it increasingly difficult to decide with certainty whether release had occurred (e.g. individual 1, 5–15 April). Sometimes, too, release appeared to be partial, and some doubt was entertained as to the exact time. The most reliable method of observation late in development was found to be as follows: the plate was held against a strong light, when the dark kidney-shaped egg mass, if present, showed up against the translucent shell. Under these conditions the ovary, not being pigmented, only slightly masked the egg masses.

A second difficulty in assessing the state of the gonads arose from infections by *Hemioniscus balani*, a parasite which lives in the mantle space where the fertilized eggs are normally found. This parasite was rife in south Devon during the period of these experiments (Southward & Crisp, 1952, 1954). When an individual first became infected no obvious change could be observed, but such individuals, though retaining ovaries, became suspect as they ceased to be fertilized for an abnormally long period. Later the condition of the ovary gradually changed, the healthy yellow or cream lobular tissue becoming amorphous and milky in appearance. When microscopically examined it was found to lack developing eggs and to be composed of diffuse fatty tissue like that of the immature ovary. At this stage the parasite could often be seen through the thin sheet of ovarian tissue as a yellow or reddish brown mass, easily to be confused with developing egg masses, but distinguishable in the records because of the sterile history of the individual carrying it. These parasitized individuals were subjected to prolonged observation, but were excluded from analyses of breeding behaviour. On no occasion was an individual bearing an obvious parasite found to be fertile, yet in spite of the relatively large size of the parasite the host did not appear to suffer otherwise. Many individuals tolerated parasites for periods up to a year. In larger species, such as *Balanus balanoides*, *B. porcatus* and *B. hameri*, this parasite usually, but not invariably, produces similar castration of the female gonad.

Definition of Terms

The reproductive cycle of a single brood occupies the time from one fertilization (oviposition) to the next; this will be called the brood period T. It may be subdivided into the fertilized period T_F which covers the time from oviposition to liberation of the nauplii, and the empty period T_E from liberation to the next fertilization and oviposition. There will also be a period of early embryonic development, recognizable from the colour change of the egg masses from cream to the brown and apparently fully developed state, which is normally shorter than the fertilized period (see p. 360); this will be called T_D.

During the breeding season there will be a certain fraction θ of the population bearing eggs, and a complementary fraction $(1-\theta)$ empty.

On the assumption that individual values of T and T_F are narrowly dispersed about their means $\overline{T}$ and $\overline{T}_F$,

$$\theta \simeq \frac{\overline{T}_F}{\overline{T}}. \tag{1}$$

This relation holds approximately only when T and T_F do not vary widely about their means, since strictly θ is equal to the mean value of (T_F/T) taken over the population, i.e.

$$\theta = \frac{1}{n}\left[\left(\frac{T_F}{T}\right)_1 + \left(\frac{T_F}{T}\right)_2 + \ldots + \left(\frac{T_F}{T}\right)_n\right]. \tag{1a}$$

The average frequency of fertilization is most conveniently defined as the proportion of individuals fertilized per day; this will be called ϕ_F. The corresponding quantity for the rate of liberation will be the proportion of individuals liberating nauplii per day, and will be termed ϕ_L. Clearly in the steady state with θ constant,

$$\phi_F = \phi_L.$$

If the brood periods are narrowly dispersed about a mean $\overline{T}$, then clearly

$$\phi \simeq \frac{1}{\overline{T}}. \tag{2}$$

If the variations in T are wide this relation does not hold very approximately since

$$\phi = \frac{1}{n}\left(\frac{1}{T_1} + \frac{1}{T_2} + \frac{1}{T_3} + \ldots + \frac{1}{T_n}\right). \tag{2a}$$

Now since the average fertilization rate ϕ_F will be dependent on the proportion of individuals, $(1-\theta)$, at that time in the unfertilized condition—since these alone are capable of being fertilized—it would be more natural to consider the fertilization rate only in relation to these individuals. Accordingly, we may introduce a fertilization constant, α, theoretically independent of θ, defined as

$$\alpha(1-\theta) = \phi_F. \tag{3}$$

Similarly, a liberation constant, independent of θ, will be given by

$$\beta\theta = \phi_L. \tag{4}$$

Thus the constant α gives the absolute rate of fertilization were the population composed entirely of unfertilized individuals; the constant β gives the absolute liberation rate on the basis of fertilized individuals only. This overcomes the difficulty which would arise in comparing average rates taken over populations containing different proportions of fertilized individuals.

Since under actual conditions and with small samples θ is not constant, the variation of θ with time will be

$$\frac{d\theta}{dt}=\phi_F-\phi_L. \tag{5}$$

Hence
$$\frac{d\theta}{dt}=\alpha(1-\theta)-\beta\theta. \tag{6}$$

Combining equations (1), (2) and (4),

$$\beta\simeq 1/\overline{T}_F, \tag{7}$$

i.e. the liberation constant approximates to the reciprocal of the fertilized period.

Combining (1), (2) and (3),

$$\alpha\simeq 1/(\overline{T}-\overline{T}_F).$$

Hence clearly
$$\alpha\simeq 1/\overline{T}_E, \tag{8}$$

i.e. the fertilization constant approximates to the reciprocal of the empty period.

SEASONAL CHANGES IN REPRODUCTIVE ACTIVITY

Table I shows that the behaviour of individuals can differ very considerably; for example, during the period covered by the table, the specimen numbered 3 gave evidence of four broods and continued to breed throughout the following summer, while number 10 showed no breeding activity till considerably later in the season. It was therefore necessary to combine the results from many individuals; in this survey the number examined varied from about 170 at the outset to 50 at the end of the experiment. This was considered sufficient to give a reliable picture of the reproductive activity of the population as a whole. The number of individuals which underwent fertilization (or liberation) between successive observations was counted, and the results presented in the form of a histogram showing the percentage of the population fertilized (liberated) per day. The recorded heights of the histogram therefore give the sample values of the average fertilization (or liberation) rate $\phi_F(\phi_L)$. The number of individuals bearing fertilized eggs was also counted and expressed as a fraction of the total population examined. The values of θ so obtained were also plotted against time (in days). The full results covering a period from August 1949 until May 1951 are shown in Fig. 2. The results were broken on 26 September 1949, when the original plates (A) were replaced by a new set (B) containing many immature individuals; this changeover is marked by a dotted line. The set of plates (B) were left out for the remainder

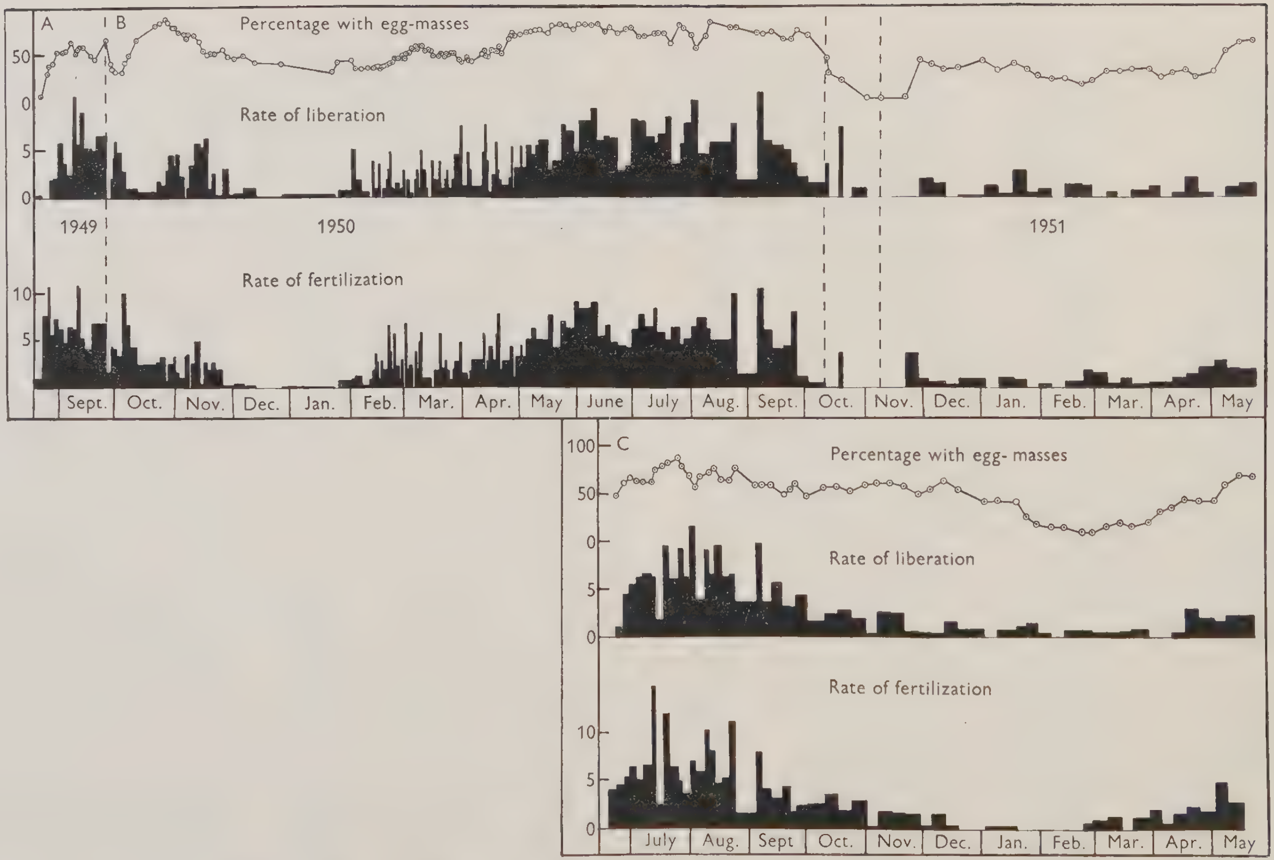

Fig. 2. Histograms showing frequency of liberation (upper) and fertilization (lower) for three series of barnacles, as percentage of population per day. Series A covers the period from August to September 1949, series B from September 1949 to May 1951, and series C, shown in the lower part of the figure, from June 1950 to May 1951. Series B were starved from October to November 1950, as shown by dotted lines. The points graphed above each pair of histograms give the percentage of individuals bearing fertilized egg masses.

of the experiment. In mid-June 1950 a further set of plates (C) were put out containing younger barnacles most of which had settled very late in the preceding year. These gave results closely similar to series B which had settled early in 1949, and are shown separately for comparison. The only noticeable difference between the B group and the younger C barnacles occurred in October and November 1950, when the B series were subjected to experimental treatment (see p. 372).

The seasonal character of the breeding activity is very clear from these results, both fertilization and liberation rates rising steadily to a maximum in mid-summer and falling to a minimum in mid-winter. In the equable climate of south-west England the breeding of continuously submerged specimens does not quite cease even in the coldest months, though it appears to do so in the normally more severe winters in south-east England. Furthermore, the rates of fertilization and of liberation (ϕ_F and ϕ_L) remain closely similar to each other at all times of year, hence apart from statistical fluctuations the proportion of individuals carrying egg masses (θ) varies only very slowly in accordance with equation (5) above.

The seasonal change in θ from a value of about 80% in summer to about 15–20% in winter is due to a slight excess in the number of fertilizations over the number of liberations throughout the spring and to the reverse occurring during autumn.

The initial changes that occur when a group of individuals come to maturity is shown best by series A which consisted of individuals of closely similar age. Maturity was reached at a remarkably early age. A few individuals were found to possess fertilized eggs within 8 weeks of settlement, just at the commencement of the records shown in Fig. 2. These individuals were about 4–6 mm. in diameter measured across the basis. From the middle until the end of August the fertilization rate was high, and since few egg masses were sufficiently far advanced in development, liberations were only occasional and θ consequently rose steeply, reaching 50% by 31 August. Thus half the population had become mature within 10 weeks of settling. Thereafter the remainder of the population matured, but since by now many egg masses had ripened the rate of liberation approached the rate of fertilization causing θ to increase more slowly. By mid-September θ was about 60–70%, a value close to that normal for fully mature populations at that season. An examination of the eggs showed that all stages of development were present and their distribution did not differ significantly from that of samples taken at random on the shore. Thus within 12 weeks of settlement this young population had not only become fully mature, but had reached a breeding equilibrium, or steady state, indistinguishable from that found in colonies of older individuals.

Fig. 3 gives the values of the fertilization constant α and the liberation constant β obtained by the application of equations (3) and (4) above and the elimination thereby of θ, the fraction bearing fertilized eggs. The sea-water temperatures and the phases of the moon are shown in the same figure. The results of series A, B and C (omitting all records where the barnacles were subjected to experimental treatments) have been combined, in order to obtain the most representative figures covering the whole period of the experiment from August 1949 until May 1951.

The constant β is inversely related to the average length of time that each individual carries its eggs ($\bar{T}_F$, eqn. 7 above), which clearly cannot normally be less than the time of embryonic development T_D (p. 361). Hence, over an extended period, β cannot be greater than the rate of development of the egg masses, though it may well be less if fully developed embryos are retained beyond full term. If release occurs at the end of embryonic development and is not determined by external stimuli, the variation in $\bar{T}_F$ and β broadly represents the temperature dependence of the physiological changes occurring during development.

The constant α depends inversely upon the average empty period $\bar{T}_E$ (eqn. 8 above) and would become very large if $\bar{T}_E$ were reduced to a very short interval of time. For this to occur fertilization must regularly take place just after release, and the ovaries must therefore have reached maturity during the fertilized period T_F. If some ovaries remain immature the empty period will be correspondingly increased and α reduced. Variations in α therefore indicate how far the re-development of the ovary keeps pace with embryonic development, high values implying that the ovaries are maturing very rapidly. This in turn indicates rapid assimilation, either through food being abundant, or because a high level of feeding activity results in much sea water being filtered.

The seasonal variation of β is seen from Fig. 3 to be clearly less than that of α, for whereas in winter β is sometimes greater than α it is always considerably less in summer, never exceeding 20 liberations per 100 individuals per day.

It follows therefore that the rate of development of embryos (nauplii), though considerably increased in summer, is not affected to the same degree as is the rate of maturation and re-fertilization of the eggs. In other words, during spring and summer the supply of food is sufficient to allow a steady output of broods of nauplii, the interval between broods being limited mainly by the time taken by the egg masses to develop. During late autumn and winter, on the other hand, the rate of assimilation appears to be the limiting factor, for α is so much reduced that in spite of the considerable retardation in embryonic development the greater part of the population remains in the empty condition. The ovaries tend to be small and poorly developed at this season, whereas the male organs, though somewhat reduced, are still present. Breeding therefore appears to be limited more by lack of food for the eggs than by any deficiency in the male organs.

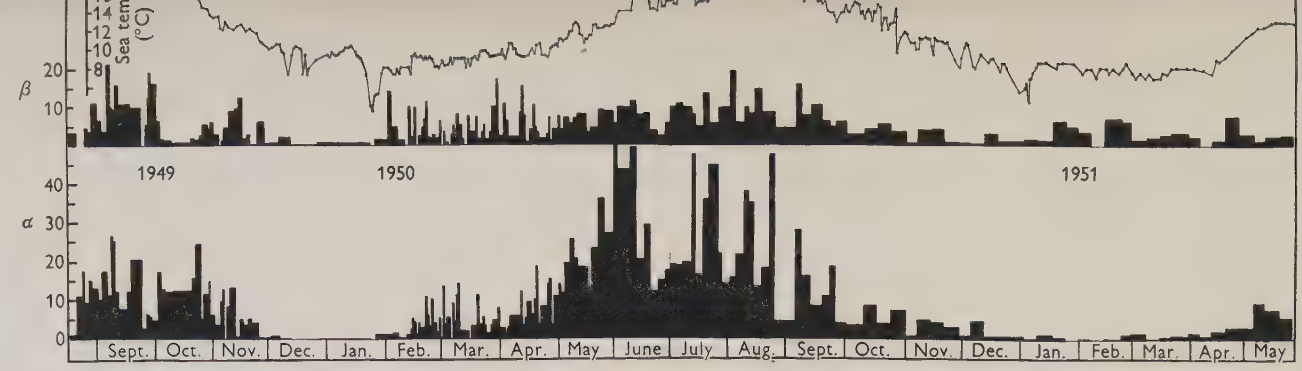

Fig. 3. Histograms showing the fluctuations in the values of the fertilization constant α and the liberation constant β over the period August 1949 to May 1951 for all series of barnacles. The sea temperatures are graphed above the histograms, and the times of maximum tides corresponding to full and new moons are shown.

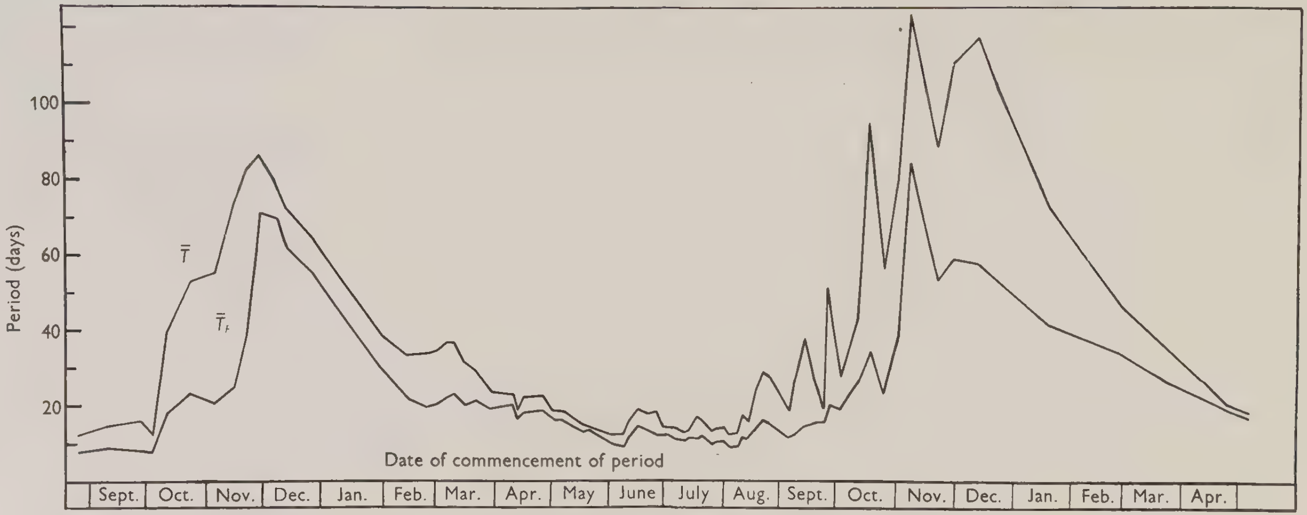

Fig. 4. Mean values of brood period $\overline{T}$ (upper), and fertilized period $\overline{T_F}$ (lower), from August 1949 to May 1951. The values of $\overline{T}$ and $\overline{T_F}$ are plotted against the date of commencement of the brood and fertilized periods respectively.

It will be noted from the histograms of α and β that whereas variations of β are approximately correlated with rise and fall in temperature, and form a roughly symmetrical pattern about the seasonal temperature maxima and minima, the values of α are higher in early than in late summer. They are also distinctly higher over the months of February–May in 1950 than in the corresponding period of 1951, which was relatively wet and sunless. The higher phytoplankton content which occurs during the first half of the year, especially when periods of sunshine are prolonged, is probably responsible for these differences. The records show that during spring the ovaries develop more rapidly than in autumn, and frequently mask the egg masses fairly early in their development (p. 360–1).

In Fig. 4 the mean values of the brood and fertilized periods ($\overline{T}$ and $\overline{T}_F$) are shown, plotted against the time at which the period commenced. In order to offset the effect of the rather wide individual variations, the mean values of T and T_F were taken over several sets of observations when few fertilizations occurred. The results show that during summer the brood period is on average about 14 days, and the fertilized period, probably representing the time of embryonic development, is about 10 days. In winter both brood and fertilized periods are much longer and fluctuate considerably, especially the brood period. This is to be expected since the rates of development and of feeding, like other physiological changes, have a high temperature coefficient and virtually cease below a critical temperature level; these processes during winter may therefore be almost entirely dependent upon short warm periods which occur from time to time. It is noteworthy that in the particularly mild winter of 1949–50 the periods $\overline{T}$ and $\overline{T}_F$ were not so extended as in the more normal winter of 1950–51, the effect of which was further prolonged by the lack of sunshine in the early spring of 1951.

The nauplius larvae hatch equally readily from the egg masses at the end of a developmental period of 10 days in summer as after one of 60–80 days in winter. Thus normal development can tolerate a very wide range of temperature.

Fig. 5 illustrates the variation between individuals in the times of development T_D, the fertilized period T_F and the brood period T, taken over short periods of about a week at different seasons of the year. The data are given as frequencies plotted on probability paper against time on the horizontal axis. By this method of presentation normal distributions appear as straight lines (Harding, 1949), with the intercept of the mean value at the 50% frequency level. It should be noted that the values of standard deviation for T and T_F obtained from Fig. 4 are not entirely due to individual variations in behaviour. They necessarily include a small error term arising from the interval between observations, which causes the measurements of T and T_F to be grouped about discrete values. This error can be obtained by Sheppard's correction in particular cases, but except where the interval between observations was a long one it was small and generally insignificant.

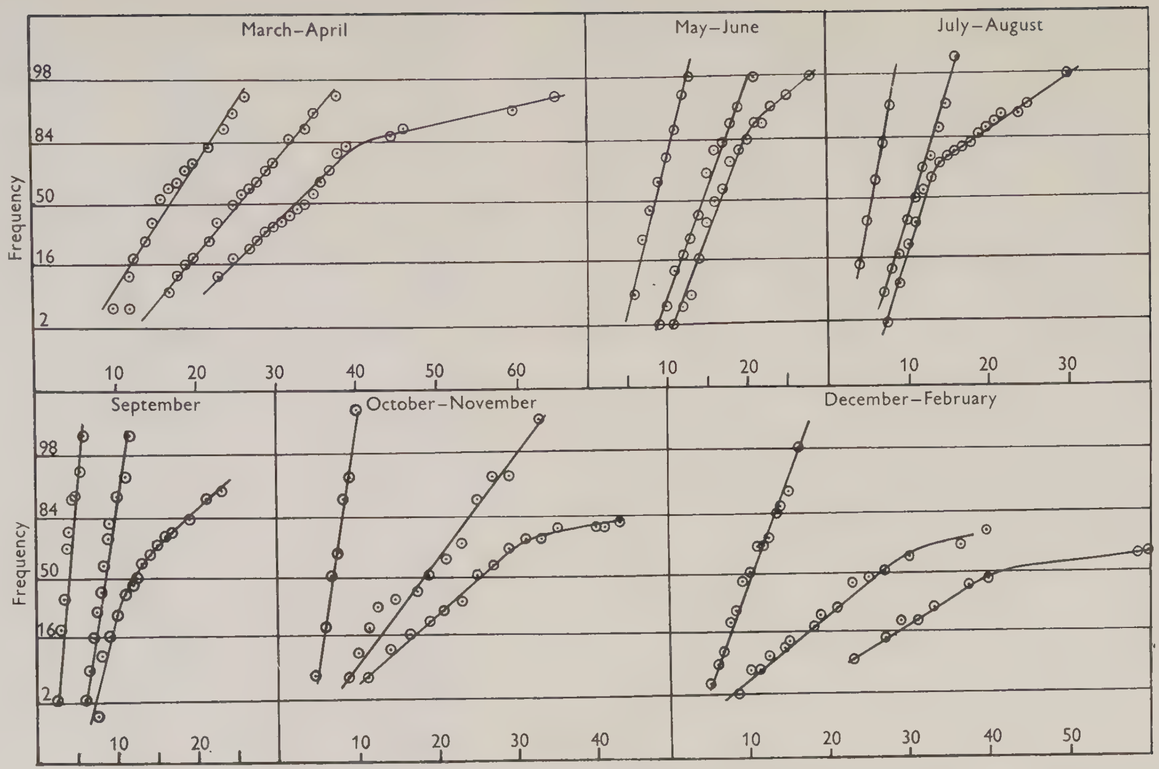

Fig. 5. Cumulative frequency curves for time of development T_D (left), the fertilized period T_F (centre) and the brood period T (right) at various seasons. The frequency axis is drawn on a probability scale, so that normal distributions are presented as straight lines. Horizontal axis gives the time in days.

The graphs reveal an interesting difference in distribution between T_D and T_F on the one hand and T, the brood period, on the other. The former distributions give very nearly straight lines and therefore are approximate to normality, whereas the longer brood periods show in every instance a marked increase in variability, the line bending towards the time axis. The explanation suggested here is based upon the view outlined above that the brood period may be limited either by the rate of development of the previously fertilized egg masses, or it may be limited by the rate of re-development of the ovary. Those individuals which are feeding more vigorously or are in more advantageous positions to obtain food will belong to the former group. They will have brood periods only slightly greater than the corresponding fertilized periods, and will fall on a distribution line not far removed from and of similar slope to that of the fertilized period. Other individuals less fortunately placed or feeding more slowly will become fertilized again only as their ovaries mature in due course. If the variability in the time of ovarian development is greater than that of embryonic development, as might well be expected, then these latter individuals will fall on a different distribution line which diverges at a lesser slope to the time axis. A perusal of the individual graphs will show that whereas in early spring and summer no more than 10 or 20% of individuals appear to belong to this divergent group, the proportion increases steadily during late summer and autumn, when food material probably becomes less readily available.

Relation between Reproduction and Temperature

Off the south-east coast (Burnham-on-Crouch) breeding occurs in *Elminius* at the highest summer temperatures (23–25° C. in some years), and survival is possible at temperatures well below zero. Our observations in the more equable climate of south Devon are limited to the range 5–18° C. If all values of α and β shown in Fig. 3 are plotted against the temperature a wide scatter results, particularly among the values of a. If the values of the reproduction constants are separated into two sets, one for the period of rising temperature and the other for the period of falling temperature, corresponding broadly to spring and autumn respectively, much of the variation is eliminated. By grouping each set of observations over limited temperature ranges the curves shown in Fig. 6 were obtained.

They demonstrate clearly the difference in the fertilization rates α for the earlier and later halves of the year after temperature has been eliminated as a variable. The relation between β and the temperature, however, can be approximated by a single line, as shown, although the points on the lower section show a tendency towards asymmetry, with β somewhat greater on the rising than on the falling temperature gradient. This would imply a tendency towards longer retention of the embryos in late autumn than at corresponding temperatures in early spring.

Fig. 7 gives the average values for the period of embryonic development, for the fertilized period, and for the total brood period, again divided into two series according to whether the seasonal temperature is rising or falling. These results are based on the individual breeding times, whereas those shown in Fig. 6 are derived from population values of the rates of fertilization and release; the values of α, however, are of the same order as $1/(\overline{T}-\overline{T}_F)$ (eqn. 8) and β fairly closely approximates to $1/\overline{T}$ (eqn. 7) at a given temperature and season.

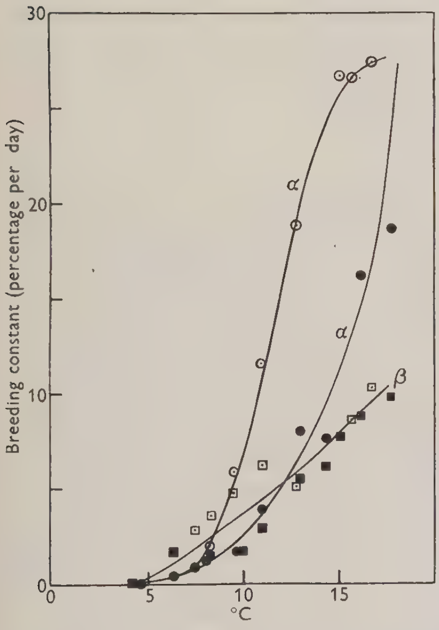

Fig. 6

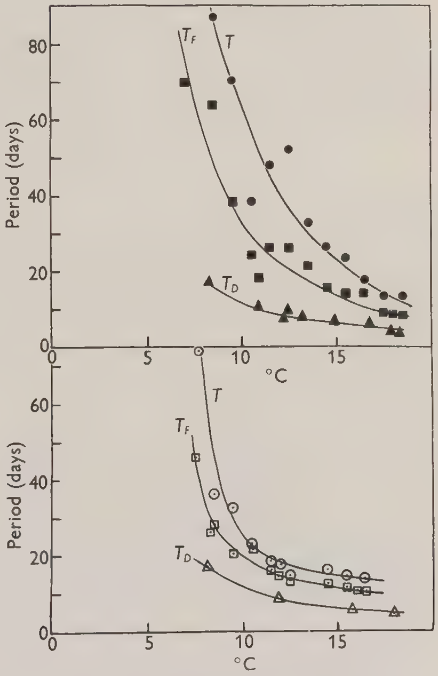

Fig. 7

Fig. 6. Temperature dependence of the fertilization constant α and the liberation constant β. ⊙ α, rising temperatures; ● α, falling temperatures; ⊡ β, rising temperatures; ■ β, falling temperatures.

Fig. 7. Temperature dependence of development, fertilized, and brood periods. Upper figure on falling, lower figure on rising temperature gradient. ○, brood period T; □, fertilized period T_F; △, time of development T_D.

The results given in Fig. 7 show rather more clearly the temperature dependence of the various processes. The period of early development T_D, given by the lowest curve in each graph, is practically identical in spring and autumn, and appears to depend only upon the temperature, as might be expected. The curves for the period of retention of embryos T_F, and for the brood period T, taken on a rising temperature gradient in the spring (lower part of figure) exhibit a relation to temperature closely similar to that of the rate of embryonic development. The values for Q_{10} based on the more reliable sections of the curves, namely those between 9 and 16° C., are 3·6, 3·3, and 3·6 for T_D, T_F and T respectively. These values are of a reasonable order for temperature coefficients of developmental processes (see Barnes, 1937). Their identity points to a common rate-governing process—that of the development of the embryos, which when completed leads to their release from the mantle cavity and then allows another fertilization to take place. The upper set of curves illustrating conditions in autumn, on the other hand, shows considerable scatter, due to the less regular individual behaviour, and gives a wide diversity of temperature coefficients—Q_{10} being 3·6, 5·4, and 5·8 for T_D, T_F and T—on account of the increasing delay in liberation and re-fertilization.

Influence of Other Factors

Nutrition

Although no attempt was made to measure the uptake of food, the effect on reproduction of withdrawing the normal food supply was investigated by placing one of the experimental plates (series B) in a well-aerated aquarium where the amount of suspended food material was not replaced. The effect on reproduction may be seen by comparing the upper series B with the lower control series C over the period October–November 1950 (Fig. 2). The barnacles removed from the sea showed a decrease in the rate of fertilization; after a short lag there was in fact complete absence of any fertilizations for a period about equal to the time that they were starved. There was a corresponding reduction in the liberation rate, but this was delayed about a month. As a result of these changes the percentage carrying eggs in the starved group fell almost to zero, but was restored to its normal seasonal level a few weeks after its return to the sea. The percentage in the control group C did not show any appreciable change during the same period. Application of the *t* test to the difference between starved and control barnacles over the period extending from the beginning of the experiment until mid-December showed that the fertilization constant α of the starved individuals was lowered (significance level = 7 %) but that the liberation constant β was not significantly altered in relation to controls.

Water Movement

The effect of water movement on reproduction was studied in a separate series of experiments. Since *Elminius* feeds on suspended material in the water, the effects of water current and population density are linked. Either may, under appropriate circumstances, limit the rate of feeding and thereby possibly the reproductive behaviour. In a population growing on a flat plate, those in the centre will be receiving water which passes across the surface of the plate, and which has probably been filtered to some extent by neighbouring individuals, whereas those growing at the sides will receive water flowing not only in the plane of the plate but also at right angles to it; moreover, they will have immediate access to unfiltered water. We may therefore regard the latter as having better access to water than the former.

A series of panels well covered with young barnacles which had settled over a short period at Burnham-on-Crouch was put out at Brixham in January 1949 and examined at different dates during the following spring. Those which approached mature size (4–7 mm rostro-carinal diameter) were sampled from positions near the centre and at the edges of the plate. It was noted at the time that those at the edges invariably showed greater growth than those at the centre.

TABLE II. ANALYSIS OF VARIANCE OF FACTORS INFLUENCING THE BREEDING OF *ELMINIUS MODESTUS*

		Mean squares		
		A	B	C
Source of variation	Degrees of freedom	Fertilized and fully developed ovary	Fertilized	Fully developed embryos
Dates and position	4	1695·6	532·1	314·8
Sizes	2	1407·9	850·7	367·5
Access to water	1	3333·1	6579·3	1146·7
First-order interaction	14	130·6	98·5	120·5
Replicates and second-order interaction (residual)	38	67·2	60·2	55·3

Two samples each of ten to twenty individuals belonging to each sub-group (i.e. of a given size range, position, and relation to water access) were examined on each date and the number of individuals falling into classes A, B, or C recorded as a percentage. Percentages were transformed to angular values, and the variation about the mean (mean square) was calculated in respect of each of the sources of variation. The combined mean square due to replication and second-order interaction was used to determine the significance of each source of variation in turn, $P=0·05$ as the limit of significance. All factors except first-order interactions had a significant effect.

The samples taken at different times in different positions were therefore grouped according to size, in order to remove this effect from the comparison. The percentage (A) having well-developed or fertilized eggs, (B) having fertilized eggs and (C) having fully developed embryos was recorded for each sample and size group. In order to make the variances homogeneous percentages (p) were converted to angles (θ), using the transformation $p = \sin^2\theta$ (Fisher & Yates, 1943, p. 50). The success of this transformation is well illustrated in the analysis of variance (Table 2) from which it will be seen that the residual variances for A, B and C are very similar. The analysis of variance shows that the fecundity, judged on criteria A, B or C, is influenced by all the main variables, date of sampling, size and access to water. Comparison of the mean values for main effects (Table 3) shows that there was the expected rise in the percentage bearing well-developed ovaries and egg masses as the season advanced. Also, individuals exceeding 6 mm in diameter were more fecund than those between 5 and 6 mm, and these in turn bred more freely than those of less than 5 mm. There was a slight but significant difference between the fronts (illuminated side) and backs of the panels, those on the darker side breeding slightly better, although the amount of algal growth was not very different on the two sides. The difference between those settled at the centre and those at the edges, however, was very pronounced, and ranked in importance with the difference between the two extreme size groups. All the results given in the table are based on samples balanced in respect of all effects and interactions other than those which are being compared. The difference in breeding attributable to variations in access to water is therefore quite independent of the greater growth rate which also takes place at the edges.

TABLE III. EFFECT OF VARIOUS FACTORS ON REPRODUCTION IN *ELMINIUS MODESTUS*

Source of variation	Details		A	B	C
Dates of settlement and examination	Settled Sept. 1948	Ex. 19. i. 49	32·7*	18·4	14·4*
		Ex. 10. iii. 49	46·9*	18·3*	9·05
		Ex. 16. iii. 49	60·9*	24·8*	5·14*
		Ex. 30. iii. 49†	83·1	72·7	17·3
Size ranges	3–5 mm		48·2**	14·5*	4·4**
	5–6 mm		53·4*	20·7*	6·9*
	>6 mm		62·7	27·5	12·8
Position†	Fronts of panels		64·3	34·2	11·8*
	Backs of panels		62·9	43·0	18·8
Access to water	Centre of panel		47·8*	10·4*	3·6*
	Outside		61·8	31·4	12·3

Mean values given as angular transformations from percentages (p). $p=\sin^2\phi$. A, proportion with fertilized or well-developed ovaries; B, proportion fertilized; C, proportion with well-developed embryos.

† Contains information additional to that included in analysis of variance.

* Indicates significant difference from entry one line below ($P<0{\cdot}05$).

** Indicates significant difference from entry two lines below ($P<0{\cdot}05$).

The significance was determined by means of a t test applied to the differences in the mean values. The residual variance of Table 2 was used to determine in each case the standard error of the mean, taking into account the number of observations contributing to the mean.

Age of individuals

In his study of breeding in *Balanus balanoides* Moore (1935) suggested that older individuals frequently showed sterility. No evidence of sterility in relation to age was found among individuals of *B. porcatus* (Crisp, 1954). Examination of glass panels bearing identified individuals of *Elminius modestus* in their third summer (1947–50) showed that at least some bore fertilized eggs and were, therefore, capable of reproducing. These individuals must have given rise to successions of broods for 3 years; at a conservative estimate they must have produced 30–40 broods in all. It seemed possible, however, that there might be a gradual slowing down in the rate of reproduction in older individuals. To test this possibility a series of panels bearing younger individuals (series C, p. 365) was observed over the same period as the older individuals of series B. Both sets were of mature age and therefore not markedly different in average size. A comparison of the reproduction constants α and β was then made, excluding the short period when the two sets of panels were receiving different treatments. The difference between the constants α for the older and younger series (B–C) based on forty-three pairs of parallel observations was $+0{\cdot}407\%$ per day, and the standard error of the difference was $1{\cdot}30\%$ per day. The observed difference of means for β was $-0{\cdot}667\%$ per day and the corresponding standard error 0·481. In neither therefore was the difference significant.

Tidal periodicity

Tidal periodicity in breeding has been established in a number of marine animals, notably in certain lamellibranchs and annelids, and in some marine algae. A test for tidal periodicity in the breeding of *Elminius* was carried out as follows. Each tidal cycle was divided about the dates midway between the largest and smallest tides into two equal parts, a spring tide period and a neap tide period. The fertilization rate ϕ_F was then averaged over each successive spring and neap tide period and the difference $\Delta\phi_F$ noted. The same treatment was applied in regard to rates of liberation ϕ_L. A t test was applied to these differences over forty-three pairs of tidal periods, with the following results:

$\Delta\phi_F$ mean difference $= -0{\cdot}0028$, standard error of difference $= 0{\cdot}211$;
$\Delta\phi_L$ mean difference $= -0{\cdot}167$, standard error of difference $= 0{\cdot}246$.

The result is therefore clearly negative, breeding occurring at all times and with equal intensity during the lunar cycle. Since these results were obtained from raft exposures where no hydrostatic changes accompanied the tide, it does not follow that tidal periodicity may not be found in barnacles which are growing on the shore. If observations are carried out on the developmental stages of the fertilized eggs found in the mantle cavity of such individuals, however, both early and late stages may be identified at any time during the greater part of the year, whether spring or neap tides prevail. There seems therefore little indication of any tidal influence on breeding in this species.

BREEDING IN *ELMINIUS* IN RELATION TO SEASON

In *Elminius* the individual cycles or broods are non-seasonal, although the rate of breeding varies with the season. It therefore differs fundamentally from those species, such as *Balanus balanoides* (Moore, 1935) and *B. porcatus* (Crisp, 1954), in which the cycle occurs only once a year. In these species the gonads show a regular seasonal change and are at any one time in a uniform state throughout the population. These species are therefore adapted to an annual rhythm, and since sea temperatures do not vary widely from year to year each part of the breeding cycle takes place within a fairly narrow temperature range. *Elminius*, on the other hand, is able to breed over very wide temperature limits, the whole cycle being capable of completion at any temperature from 6 to 20° C, and probably higher. Even at temperatures below 6° C embryonic development may continue, but it is extremely slow.

There is in *Elminius* no evidence of any pace-making mechanism to the breeding cycle, the population at all times having a random selection of embryonic and ovarian developmental stages. Indeed, the observed variations in rates of fertilization and liberation were never greater than would have been expected from chance. The breeding rates are consistent with the view that they are entirely dependent upon temperature and food supply. The possibility that there is a seasonal rhythm superimposed is unlikely, for when young individuals were transferred from Burnham-on-Crouch to Brixham in autumn or winter they bred earlier in the south-west. Moreover, there is no definite cessation of breeding, but rather a slowing down or pausing when the temperature falls.

The concept of a fertilization constant (α) and a liberation constant (β) as defined above can be employed only in animals which reproduce by a continuous succession of broods, as for example *Elminius* and *Chthamalus stellatus*. These constants are useful not only in determining the influence of temperature and other variables, but they also give information on the mechanism of breeding. In *Elminius* it has been shown that α depends mainly upon the food supply, while β is determined by the rate of development of the eggs in the mantle cavity. This condition must result from the physiological mechanisms which control breeding. The ovary appears to develop as rapidly as assimilation can provide for it, and is frequently ahead of the development of the eggs; when this occurs liberation is immediately followed by copulation and oviposition, provided there is another individual in the vicinity to function as a male. It is noteworthy that oviposition never occurs until the previous brood is liberated. If, as seems probable, oviposition is dependent upon copulation, either there is a refusal on the part of the gravid barnacles to allow copulation, or else individuals acting as males require some stimulus which is provided only by individuals which have liberated the previous brood. Some such mechanism seems to be general, for only one brood of eggs is found in the mantle cavity of *Balanus improvisus*, *B. amphitrite*, *B. crenatus* and *Chthamalus stellatus*, all of which exhibit continuous breeding. The only exception noted by one of us (D. J. C.) was the presence of two pairs of egg masses in one or two out of several thousand specimens of *C. stellatus*, where the earlier egg masses were already fully developed, and the later egg masses only just deposited. Since *Chthamalus* lives high up the intertidal zone it may only infrequently be submerged, and it is possible that one brood might be retained beyond the normal physiological time limit. Alternatively, the removal of a specimen with very ripe ovaries might induce a false spawning of unfertilized eggs which would not be readily distinguishable from a true fertilization. Both exceptional specimens were taken at the peak of the breeding season in a situation where the ovaries were developing very rapidly.

It appears from our observations that liberation occurs soon after the eggs are fully developed. It involves activity on the part of the parent, for the eggs are discharged forcibly through the aperture between the opercular plates. Liberation of eggs does not appear to depend primarily upon the condition of the ovary of the parent, for it continues normally in specimens which have been starved and which have no new ovary developing. There was, however, some evidence that such individuals may retain the eggs a little longer, particularly at low temperatures, in that the value of β was lower and the fertilized period T_F distinctly longer on a falling temperature gradient than on a rising one.

We have assumed that the effect of the falling temperature gradient is ultimately attributable to diminished food supply in autumn and winter, leading to poorer ovarian growth and a decrease in the percentage of gravid individuals. It is equally possible that the food supply is there, but that on a falling temperature gradient the rate of feeding or perhaps the whole metabolic process is depressed by temperature adaptation.

Evidently *Elminius* is capable of being fertilized at any time of year as soon as the ovaries have regenerated. Other barnacles, particularly northern forms, show a delay after the regeneration of the ovary, fertilization only being possible at certain seasons (Crisp, 1954).

Fecundity of *Elminius* in Relation to its Ecology

The breeding behaviour of *Elminius* is well suited to its environment, namely sheltered coasts and estuaries. The remarkable eurythermy of its reproduction is clearly adapted to the wide fluctuations of temperature which occur in such habitats. Moreover, land-washed nutrients normally allow a high level of production to be maintained in enclosed waters throughout all but the coldest months. *Elminius* therefore has available abundant suspended food material for purposes of reproduction throughout most of the year. The continuous succession of broods necessitates intensive feeding, and it is noteworthy that *Elminius* has a faster rate of beat of the cirri than is found in most of the other British species, and far more rapid than that of *Balanus balanoides*. The advantage of feeding with access to unfiltered water, not only to growth but also to reproductive rate, has been shown. This effect is very pronounced in individuals exposed to a fast-flowing stream, as for example those attached to piles standing in a narrow part of a tidal estuary. Such individuals grow rapidly to a large size (1·0–1·5 cm) and a high percentage are fertile in the warmer months. The influence of water current on growth rate renders size a poor criterion of age, and large individuals cannot necessarily be regarded as having been settled for a long period. When intense settlements occur in situations exposed to strong currents, growth may be restricted laterally, the result being columnar individuals with narrow parietes tapering towards the basis. Columnar growth is less evident, however, than in *B. balanoides* under comparable conditions.

The remarkable fecundity of *Elminius* brought out in this paper is largely responsible for its rapid colonization of new shores in Britain and Europe (Crisp & Chipperfield, 1948). Assuming a span of life of three breeding seasons, and an average number of 500 nauplii in each brood, and twelve broods per season, the total output of young approaches 20,000 per individual. This is equivalent to the output over the same period of three broods of a large specimen of *Balanus balanoides* which would occupy a considerably greater area. Moreover the generation time of *Elminius* is very short compared with that of *Balanus balanoides*, which leads to a potentially much greater rate of multiplication. Taking a value of 10 weeks for the period of growth from settlement to maturity in summer (p. 365) and an assumed value of 4 weeks of planktonic life, the total generation time is only 14 weeks, compared with 1–2 years in *B. balanoides* (Moore, 1935). The generation time of *Elminius* maturing over the cooler months will of course be greater.

Another advantage held by *Elminius* over *Balanus balanoides* consists not only in the greater number of embryos produced per unit area of substratum, but also in the greatly extended period over which they settle. Under the very crowded conditions which frequently obtain in estuaries and shores where *Elminius* abounds, new individuals can only survive if they settle in the spaces from which old ones have broken away. Hence the long settling period of *Elminius* offers ample opportunities for the colonization of bare areas, whereas *Balanus balanoides* can only occupy such spaces as are available during the four weeks or so in which it settles. On the other hand, since *B. balanoides* settles about a month before *Elminius* it can take advantage of sites left available by losses during the preceding winter months. The disparity is, therefore, not as great as might be anticipated from the difference in the lengths of the settling periods. To some extent, too, the very intense settlements of *Elminius* which occur in mid-summer in shallow estuaries, such as the Crouch and Blackwater, fail to benefit the species since they produce many small individuals which retard each other's growth and mature only slowly except where conditions such as exposure to strong water currents are especially favourable.

We are indebted to Mr A. H. Molesworth who carried out some of the observations for us on occasions when we were unable to visit the raft, and to Mr P. Bowles for the drawing of Fig. 1.

SUMMARY

A more complete study of reproductive behaviour is possible where observations can be made on individuals. This is especially so in a population where each individual has a succession of breeding cycles which are not synchronous with those of other individuals. Continuous records of the breeding of individual barnacles is possible in species which have a membranous base. Individuals may be grown on glass slides and the reproductive condition determined by observations through the base.

Reproduction in *Elminius modestus* takes the form of a succession of breeding cycles or broods, each cycle being initiated by copulation, oviposition and fertilization of the eggs in the mantle cavity. Here the eggs develop, and embryos are eventually liberated through the opercular valves.

The time interval occupied by a brood varies both among individuals and with the season of the year. The time of development of the embryos appears to be a function of temperature alone, but the regeneration of the ovary depends upon the nutrition and food supply. When the ovary regenerates rapidly, as in the period of rising temperature, viz. in spring and early summer, another fertilization follows closely upon liberation, but in autumn and winter the ovary may not mature for some time after liberation, with the result that a large proportion of the population do not contain egg masses. In spring and summer the fecundity is probably limited by the rate of development of the embryos; in autumn and winter by nutrition.

There is a slight tendency for eggs to remain in the mantle cavity for a longer period during the falling temperatures of autumn and winter than at corresponding rising temperatures in spring and summer. It is possible that a rapidly maturing ovary exerts some stimulus accelerating liberation; nevertheless, liberation can take place without ovarian regeneration.

Access to previously unfiltered water, and to rapidly moving water increases both the rate of growth and the fecundity.

Older individuals do not differ significantly from young mature individuals in their breeding behaviour, unless infected by *Hemioniscus balani*, which prevents maturation of the ovary and so renders *Elminius modestus* sterile. Parasitized individuals may live for at least a year without showing any other symptom.

There is no lunar periodicity in the breeding cycle.

The breeding of *Elminius* is characterized by extreme eurythermy. It can breed at any season, provided that the temperature does not fall below 6° C and the food supply is adequate. This type of breeding is well suited to life in shallow estuaries and sheltered coasts of temperate latitudes and accounts for its success in Britain in competition with the indigenous *Balanus balanoides*.

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ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

STUDIES ON *LYSMATA SETICAUDATA* RISSO (CRUSTACEA DECAPODA). VIII. THE LACK OF INFLUENCE OF EYESTALK ABLATION AND OF INJECTION OF EYESTALK EXTRACTS ON TESTICULAR WEIGHT AND DEGREE OF DEVELOPMENT OF THE MALE GENITAL DUCTS

By **D. B. Carlisle**

Pubbl. Staz. zool. Napoli, Vol. 25, 1954, pp. 241–5

Eyestalk ablation and an injection of eyestalk extracts has no effect on testicular weight or on degree of development of the male genital ducts in *Lysmata seticaudata*. This result is quite opposite to that found by Démeusy in the crab *Carcinides*. D. B. C.

THE EARLY DEVELOPMENT STAGES OF THE BASS, *MORONE LABRAX* (L.)

By **L. A. J. Jackman**

Proc. Zool. Soc. Lond., B, Vol. 124, pp. 531–4

On 21 May 1952 some of the bass in the Plymouth Aquarium spawned, and a number of these eggs were collected and their development observed.

The eggs of the bass in British waters are larger than those in the Mediterranean, and hatching occurs on the fourth day. The mean length of newly hatched larvae is 3·83 mm.

During the first 24 hr these post-larval stages rest for long periods at the surface of the water, are insensitive to touch and no evasive action is taken.

On the third day the larvae rest at an angle of forty-five degrees to the surface. By the sixth day they commence to swim, and make rapid darts through the water easily avoiding the approach of a pipette.

By the tenth day the oil globule is partially absorbed.

Throughout these observations the mean temperature of the tanks was 15° C.

From the observed rate of growth of these reared larval stages, it is likely that those taken in the plankton hauls off the Eddystone during the period 1925–33 (Russell, 1935) were from 3–15 days old. L. A. J. J.

THE EFFECT OF GALVANIC POLARIZATION ON THE IMPULSE DISCHARGE FROM SENSE ENDINGS IN THE ISOLATED LABYRINTH OF THE THORNBACK RAY (*RAJA CLAVATA*)

By **O. Lowenstein**

J. Physiol. Vol. 127, 1955, pp. 104–17

The effects of galvanic polarizing currents on the impulse discharges from the crista of the horizontal ampulla and from other labyrinthine end-organs show that galvanic polarization produces impulse responses similar to those occurring on natural rotatory stimulation. The responses from single sensory units of the horizontal semicircular canal to galvanic stimulation by ascending and descending currents sum with the responses to ipsilateral and contralateral accelerations. This result is held to provide circumstantial evidence for the existence of a so-called generator potential intervening between the mechanical deformation of the sense organ and the propagated impulse discharge in the sensory nerve. O. L.

THE PLANKTONIC DECAPOD CRUSTACEA AND STOMATOPODA OF THE BENGUELA CURRENT. PART I. FIRST SURVEY, R.R.S. *WILLIAM SCORESBY*, MARCH 1950

By **Marie V. Lebour, D.Sc.**

Discovery Reports, Vol. 27, 1954, pp. 219–34

Most of the specimens are larvae. Among the few adults were several specimens of the Pasiphaeid *Pasiphaea semispina* recently described by Holthuis (1951–52). The present records show that it occurs much farther north than those found previously in the South Atlantic. Other interesting finds are a late larva of a *Periclimenes* with a long antennular flagellum and a peculiar pagurid larva of a type unknown before. Phyllosoma larvae of *Jasus lalandii* are common and at times a *Callianassa* larva is abundant which does not appear to belong to any known adult (the adults, always difficult to find, are very little known from their regions). Brachyura zoeae are abundant at times but not attributable to known species, the only Stomatopod present is a *Squilla* larva, almost certainly *Squilla armata* as it occurs in the same locality as the adult and agrees with it in most essential features. M. V. L.

THE PELAGIC MOLLUSCA OF THE BENGUELA CURRENT. PART I. FIRST SURVEY, R.R.S. *WILLIAM SCORESBY*, MARCH 1950. WITH AN ACCOUNT OF THE REPRODUCTIVE SYSTEM AND SEXUAL SUCCESSION OF *LIMACINA BULIMOIDES*

By **J. E. Morton**

Discovery Reports, Vol. 27, 1954, pp. 163–200

This paper records the occurrence and distribution in the Atlantic Ocean off south-west Africa of the following species of molluscs: *Ianthina ianthina*, *I. globosa*, *Atlanta peroni*, *Limacina inflata*, *L. bulimoides*, *Diacria trispinosa*, *Euclio pyramidata*, *Cavolinia inflexa*, *Cymbulia peroni*, *Thliptodon diaphanus*, *Pneumodermopsis paucidens*, as well as two species of larval lamellibranchs, one prosobranch larva, and several larval cephalopods. The ecology of *Limacina bulimoides* is discussed and information presented on its diurnal depth migrations. A size-depth division of the population appears to take place at night.

The reproductive system and sexual succession of *Limacina bulimoides* is described in detail. The genital ducts are typical of the lower level of both the groups Opisthobranchiata and Pulmonata, and in the condition of the gonad, as well as the development of penis and prostate, *Limacina* shows protandrous hermaphroditism. Six sexual stages are distinguished, ranging from small sexually undifferentiated individuals, through various stages of male development, to the females, which are the largest. A short discussion is added, dealing with the problem of the evolution of sexual succession in gastropods as a whole.

J. E. M.

CHEMICAL EVIDENCE ON THE ABUNDANCE AND BOTANICAL COMPOSITION OF THE MARINE PHYTOPLANKTON

By **W. R. G. Atkins**

Proc. 7th Int. Bot. Congr. Stockh., 1950, p. 262 (published 1953)

The sea twenty miles off Plymouth is representative of a large area subjected to uniform meteorological conditions and not normally affected by water movements from areas under other conditions. Figures for the phytoplankton crop, calculated for a depth of 70 m. were concordant when based upon the changes in phosphate, carbon dioxide and nitrate, but with silica the values were over 10 times too small. The diatom tests do not dissolve or do so slowly, but suspended clay amounts to about one part per million, and this goes into solution in measurable amounts as shown by storing in polythene bottles for three months.

Productivity may also be followed by chlorophyll estimation after filtration through collodion discs or using fine paper after addition of aluminium sulphate. In the gel thus produced small flagellates can be seen under the microscope, in slow motion. Dr M. Parke's pure cultures afforded counts, which, excluding large diatoms, ran from 50 to over 3000 million cells per milligram of chlorophyll. W. R. G. A.

MECHANICAL STIMULATION IN THE SEA-ANEMONE *CALLIACTIS PARASITICA*

By **L. M. Passano** and **C. F. A. Pantin**

Proc. roy. Soc. B, 1955, Vol. 143, pp. 226–38

A method of administering measured local mechanical stimuli is described. Experiments were done upon the anemone *Calliactis parasitica*.

Mechanical stimuli show rapid apparent adaptation—partly due to simple mechanical causes such as contracture and passive deformation of the tissues.

When conditions are standardized a mechanical stimulus of sufficient intensity on the column gives a nervous impulse. These mechanical stimuli can be used in the same way as electric shocks to give facilitated responses.

Increasing the mechanical intensity shows (*a*) a threshold below which no impulse is generated, and (*b*) with further increase of strength, trains of increasing numbers of impulses.

There exists a graded mechanically excitable system. Gradation is to be observed below the threshold since there can be summation of subliminal impulses.

The excitable system appears to be orientated tangentially and responds to strains rather than pressure. The excitable system is purely endodermal. The ectoderm and mesogloea act only as an integument.

On histological grounds there are grave difficulties in supposing that impulses arise simply and directly in the numerous simple sense organs. The possibility is noted that the graded excitation of the mechanically sensitive system causes the nerve-net to fire off impulses.

The sensitivity of different parts of the animal varies greatly. In *Calliactis* the oral disc is at least 4000 times as sensitive as the column. The combination of a simple mechanically excitable system with the gross morphological features of the bodily organization permits purposive and varied responses. Thus strong stimulation of the column leads to the closure reflex, whilst weak stimulation of the disc by contact or by water movements leads to appropriate responses connected with feeding or rejection. C. F. A. P.

RECHERCHES PRÉLIMINAIRES RELATIVES À LA SEPARATION ET À LA COMPARAISON DES SUBSTANCES CHROMACTIVES DES CRUSTACÉS ET DES INSECTES (PRELIMINARY STUDIES ON THE SEPARATION AND COMPARISON OF THE CHROMACTIVE SUBSTANCES OF CRUSTACEA AND INSECTS)

By **David Carlisle, Mme. Marie Dupont-Raabe and Sir Francis Knowles**

C.R. Acad. Sci. Paris, T. 240, 1955, pp. 665–7

The chromactive substances of the sinus gland and post-commissural organs of crustacea and of the brain and *corpora cardiaca* of insects were separated by paper electrophoresis. The most striking results were the individuation and characterization of various chromactive substances. The first, designated substance A, was found in all these organs except the insect brain. It provoked concentration of all the red and yellow pigments of *Leander*. It was stable to boiling and did not pass freely through dialysis membranes. The second substance, substance B, was found only in the post-commissural organs. It concentrated the large red chromatophores of *Leander*, but expanded the small red chromatophores of the body and tail. These two substances were antagonistic. The brain of the insect contained a different chromactive substance, designated substance C. This substance caused darkening of *Carausius*,but had no effect on the pigments of *Leander*. It did, however, cause concentration of the chromatophores of *Crangon*. Other more mobile substances were found in the various extracts; they all pass freely through dialysis membranes and each acts only on a single pigmentary effector type. These substances appear to be the definitive hormones while substances A and B are probably the precursors.

Extracts of the X-organ of *Leander* have no effect when fresh, but after boiling, or after alcohol treatment, they attain an activity comparable to that of the sinus gland. It is probable that the site of production of the precursors of the hormone of the sinus gland is in the X-organ. D. B. C.

J. Mar. biol. Ass. U.K. (1955) 34, 387–401
Printed in Great Britain

R.V. *SARSIA*

A BRIEF DESCRIPTION OF THE MARINE BIOLOGICAL ASSOCIATION'S NEW RESEARCH VESSEL

By G. A. Steven, C. A. Hoodless, R. S. Harrison and F. J. Warren
The Plymouth Laboratory

(With Plates I–IV and Text-figs. 1–5)

From 1922 until the outbreak of the 1939–45 war, the Association's Laboratory at Plymouth was well served by the research vessel *Salpa*—a converted Lowestoft steam drifter having an overall length of about 95 ft. In 1939 *Salpa* was taken over by the Admiralty for naval duties and was not put back into service by the Association on her release after hostilities ended. Her place was taken by a 90 ft. motor fishing vessel on charter from the Admiralty and named *Sabella*. This vessel was only partially converted for research work and was handed back to her owners when *Sarsia* came into service at the end of 1953.

In consultation with the Laboratory staff, *Sarsia* was designed by Messrs Graham and Woolnough, Liverpool, and built by Messrs Philip and Son, Ltd., Dartmouth, to Lloyds 100 A1 classification 'Motor Trawler'. Much thought went into the planning of a ship that would be able to cope successfully and economically both with short trips to near-by grounds to collect and bring back living animals and with extended cruises to more distant and deeper waters. To meet the former requirements the vessel must not be too large and unhandy for inshore dredging and trawling: for the deeper work it was essential that she be large enough to stand up to heavy weather and provide a comfortable 'platform' in all reasonable conditions.

It early became obvious that the restriction on size ruled out steam—in spite of its many virtues—as a medium of propulsion. The cost of maintaining banked up fires at night while the vessel is employed in daily collecting was also a consideration. In spite of its various shortcomings, diesel propulsion was finally decided upon.

General Plan of the Ship

Hull. The hull is of riveted steel construction. Principal dimensions are:

Length overall 128 ft.	Tonnage gross 319
Breadth moulded 28 ft.	Tonnage net 95
Depth moulded 13 ft.	Designed speed 10 knots
Mean load draft 9 ft. 6 in.	

The general appearance of the vessel is clearly shown in Plate I. She has a soft-nosed raked stem and a cruiser stern. The main deck is continuous fore and aft carrying a forecastle forward and a large steel deck-house, the after part of which provides a spacious boat deck. At the forward end are the wheel-house, chart-room and master's accommodation. The wheel-house is raised 3 ft. above the level of the boat deck to provide a good view forward over the forecastle head.

Forecastle. An electric windlass is fitted on the forecastle deck. This supplies power for working the two bower anchors (about 7 cwt. each) and 105 fm. of $1\frac{1}{16}$ in. chain cable—60 fm. on the starboard anchor and 45 fm. on the port one. This cable is stowed in a locker below the main deck. In the forecastle are situated the paint locker, lamp-room, scientific store and main hydrographic winch which is completely enclosed. When this winch is in use a door in the forecastle bulkhead is opened and gives the winch operator a clear view of the hydrographic davit. The scientific store[1] is fitted with shelves for stowage of collecting jars, rails for hanging up small collecting nets, and has ample deck space for storing dredges, Agassiz trawls, and similar deck gear. It also contains a work bench and vice. A large opening gives access to the store from the main deck. This opening is fitted with pound boards which may be used completely to close the opening or to make a 'water step' of any desired height.

Main deck. The space between forecastle and deck-house is mostly occupied by trawl fitments. The winch is normally sited, immediately forward of the deck-house. It is fitted with three drums, the centre and starboard one each carrying 500 fm. of $2\frac{1}{2}$ in. wire, while the port drum carries 1800 fm. of $1\frac{3}{8}$ in. wire. The trawling fair-leads are so arranged that any wire from the winch can be led to either gallows or to the main derrick on the foremast. Two of the fair-leads are fitted with mechanisms for recording length of wire out in both fathoms and metres—one for the after trawl warp and one for the long small general-purpose wire on the port drum. On the port side of the foredeck a 'Discovery' type hydrographic davit is fitted, and a small hatch with a steel cover provides access to the deck below. The foremast is stepped on this deck and carries a derrick tested to 5 tons safe working load.

The deck on the starboard side of the main deck-house is kept clear for trawl handling. On the port side are two fixed tanks with hinged lids and provided with a continuous supply of salt water. Portable tanks are similarly supplied with running sea water from a supply pipe fitted along the bulwarks.

For a distance of 18 ft., between the after end of the deck-housing and the stern, the deck is clear, providing a most useful working space when gear is being used over the stern or quarter.

Main Deck-house. This structure, 60 ft. long and 15 ft. wide, extends from immediately abaft the trawl winch to within 18 ft. of the stern. The main laboratory (mainly hydrographic) is located in the forward end. From this laboratory a door opens aft into a passage-way which extends across the house

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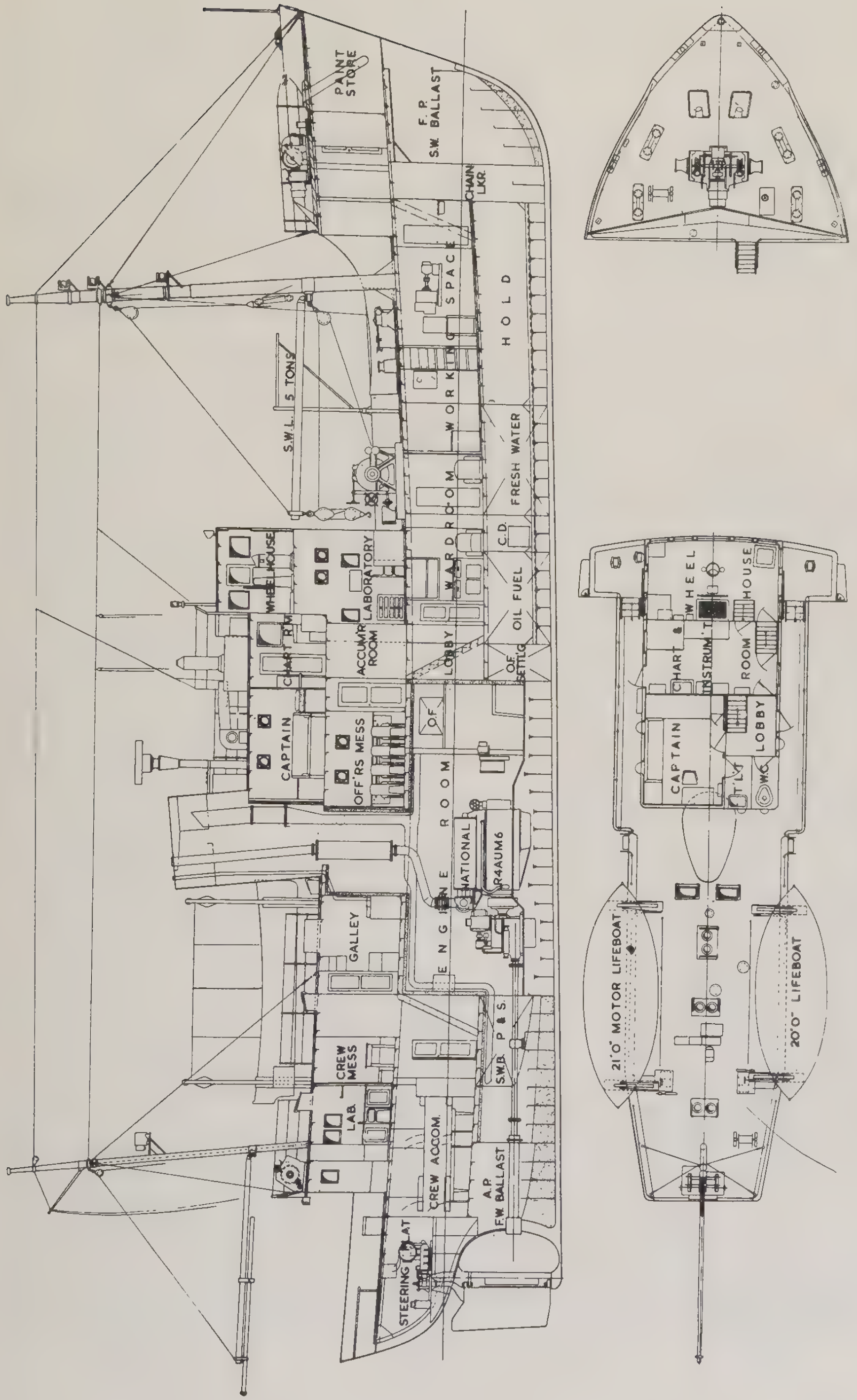
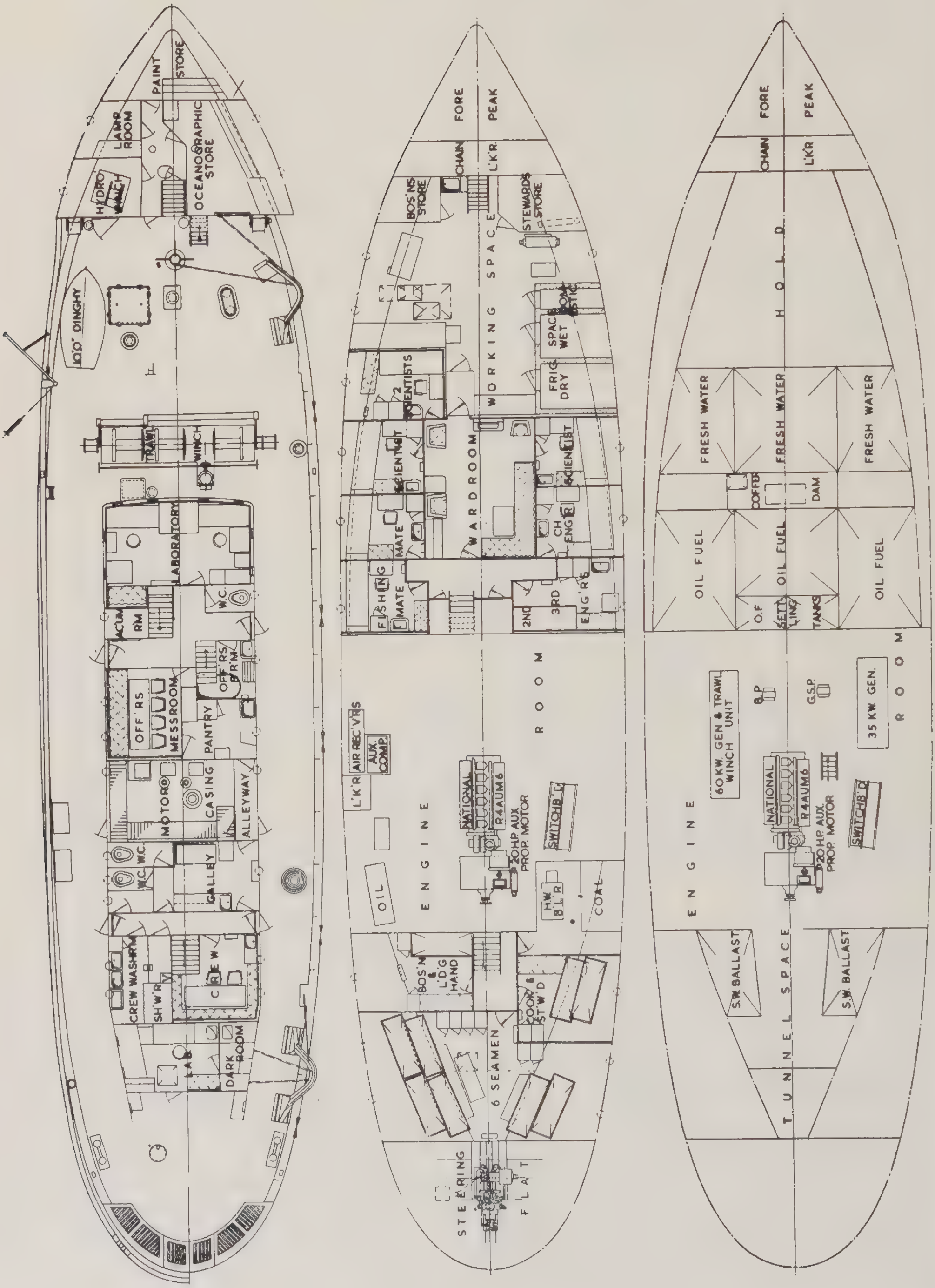
[1] Incorrectly named 'oceanographic store' in the builders' drawing in text-fig. 2.


PAINT STORE
F.P. S.W. BALLAST
CHAIN LKR.
WORKING SPACE
HOLD
S.W.L. 5 TONS
FRESH WATER
WARDROOM
C.D.
OIL FUEL
WHEELHOUSE
LABORATORY
CHART RM
ACCUMR ROOM
LOBBY
O.F. SETTLG
O.F.
CAPTAIN
OFF'RS MESS
ENGINE ROOM
NATIONAL
R4AUM6
GALLEY
P & S.
S.W.B.
CREW MESS
LAB.
CREW ACCOM.
A.P. F.W. BALLAST
STEERING FLAT
WHEEL HOUSE
CHART & INSTRUM'T ROOM
CAPTAIN
LOBBY
T'LT
W.C.
21'0" MOTOR LIFEBOAT
20'0" LIFEBOAT

Text-fig. 1. Profile section and general arrangement plan of bridge, boat deck and forecastle deck.


PAINT STORE
LAMP ROOM
OCEANOGRAPHIC STORE
HYDRO WINCH
10'0" DINGHY
TRAWL WINCH
LABORATORY
W.C.
ACUM RM
OFF'RS B'R'M
OFF'RS MESSROOM
PANTRY
MOTOR CASING
ALLEYWAY
W.C.
W.C.
GALLEY
CREW WASH RM
SHW'R
CREW
LAB
DARK ROOM
FORE PEAK
CHAIN L'K'R
BOS'NS STORE
STEWARDS STORE
WORKING SPACE
DOMESTIC
WET SPACE
FRIG DRY
2 SCIENTISTS
SCIENTIST
WARDROOM
SCIENTIST
CH ENG'R
MATE
FISHING MATE
2ND
3RD ENG'R'S
L'K'R
AIR REC'V'RS
AUX. COMP.
NATIONAL R4AUM6
20HP AUX PROP. MOTOR
SWITCHB'D
ENGINE ROOM
OIL
H.W. B'L'R
COAL
BOS'N & L'D'G HAND
COOK & ST'W'D
6 SEAMEN
STEERING FLAT
FORE PEAK
CHAIN L'K'R
HOLD
FRESH WATER
FRESH WATER
FRESH WATER
COFFER DAM
OIL FUEL
OIL FUEL
OIL FUEL
O.F SETTLING TANKS
60 KW. GEN & TRAWL WINCH UNIT
B.P
G.S.P
35 KW. GEN.
NATIONAL R4AUM6
20HP AUX PROP. MOTOR
SWITCHB'D
ENGINE ROOM
S.W. BALLAST
S.W. BALLAST
TUNNEL SPACE

to doors opening on to the main deck (port and starboard). From this passageway direct access is obtained to the officers' and scientists' messroom, bathroom and W.C.; and to ladders leading up to the wheel-house and down to the deck below.

The messroom, comfortably fitted and provided with the usual appointments, connects directly with a steward's pantry containing a domestic refrigerator, hot cupboard and other usual fitments.

Abaft the messroom and pantry is the engine-room casing, along the starboard side of which an alleyway extends aft to the galley. Its cooking range is of the old-fashioned coal-fired type. Despite objections to carrying coal as an additional fuel, this type of range is welcomed by all as providing the only warm and comfortable spot on board when all the ship's machinery is 'dead', and no shore electrical supply is available.

An athwartships alleyway abaft the galley gives access to the deck, port and starboard. On the port side, forward of this alleyway and opening off it, are crew's toilets and an oilskin locker. Abaft the alleyway, also on the port side, is the crew's wash-place—containing two ordinary hand basins, a trough, and a shower—and a drying room. From the centre of the alleyway a ladder leads down to the crew's sleeping quarters. Abaft the alleyway, on the starboard side —i.e. directly opposite the galley—is the crew's messroom.

In the after end of the deck-housing with a door opening aft, is a second small laboratory—mainly biological—part of which is partitioned off to form a fully fitted dark-room. Abaft this laboratory a small section of the open deck is sheltered by an extension overhead of the boat deck and a corresponding continuation of the casing on each side.

Bridge Arrangements. These arrangements are rather unusual and contribute greatly to easy working. The wheel-house, being raised 3 ft. above the level of the chart-room, provides easy access to the flying bridge which is sited directly above the chart-room. The flying bridge carries the standard compass, radar scanner, ventilating fans and gravity tanks. A wood screen around the fore-end provides adequate shelter.

The wheel-house (Pl. III A), in addition to basic fitments, contains a Decca Radar Unit Type 12, a Marconi 'Graphette' echo-sounder, a Walkers 'Trident' electric log recorder, a telephone, an electric rudder indicator, and an engine-revolution indicator. The port forward window is fitted with a Kent's clear-view screen. Sliding doors, port and starboard, give access to open bridge wings which are carried on sponsons. An engine-room telegraph is fitted on both wings and a revolution telegraph on the port wing. A portable searchlight can be mounted and worked on either wing.

From the starboard side of the wheel-house a few steps lead down into the chart-room (Pl. III B). Across the forward end of this space is a chart table and a long writing table with drawers and cupboards under. The forward bulkhead carries two Kelvin and Hughes echo-sounders—one Type MS 26B

(0–720 ft. or fm.) and one MS 26E (0–2250 fm.). A Decca Navigator decometer unit is fitted to the bulkhead just above the table level. In the cupboards under the table converter units for the echo-sounders and radio-telephone are housed.

On the after bulkhead, the Decca Navigator receiver panel, Redifon radio-telephone and Decca radar receiver panel are fitted. A door opening aft on the starboard side leads into a lobby giving access to the Master's quarters. From this lobby a ladder leads down to the main deck.

Boat Deck. The funnel is oval (Pl. II B) in cross-section and merges into the after part of the Master's accommodation. Abaft the funnel there is, on the starboard side, a 20 ft. standard life-boat and on the port side a 21 ft. motor life-boat fitted with an 8 h.p. Stewart Turner petrol engine. Near the after end of the boat deck the mainmast is stepped. This carries a light derrick which has a sliding boom fitted along its underside. This boom can be slid outboard to obtain up to 8 ft. clearance for light gear worked over the ship's stern or quarter. Abaft the mainmast is a small two-drum hydrographic winch, one drum carrying 500 m of 4 mm (diam.) wire and the other carrying 300 m of 7 mm (diam.) wire.

Lower Deck. Abaft the chain locker on this deck is a large compartment used as a general workroom and store. On the starboard side it contains three cold rooms—one for domestic use and the two others for scientific purposes. There is also a work bench. The after bulkhead, port side, carries a battery of racks for storage of collecting boxes of special design (see p. 396). A small hatch in the port side of this deck opens into the hold space below.

From the working compartment a door leads aft into a comfortably furnished 'wardroom'. Three scientists' cabins open off the wardroom—two single-berth cabins and one double-berth cabin, all with fitted basins supplied with hot and cold running water. Abaft the wardroom is a lobby giving access to three single-berth cabins for ship's officers (mate, fishing mate and chief engineer) and one two-berth cabin shared by engineers II and III. From this lobby, too, a ladder leads up to the deck above, emerging into the alleyway abaft the main laboratory.

Crew's Quarters. The space abaft the engine-room is taken up by the crew's sleeping accommodation. This consists of a double-berth cabin for the cook and steward on the starboard side; a similar cabin for the bosun and leading hand on the port side, and a large central six-berth cabin in the after end of the ship.

The crew numbers sixteen—master, mate, fishing mate, chief engineer and two other engineers, bosun, leading hand, cook, steward and six seamen.

MACHINERY

Main engine. The main propulsion unit consists of a vertical 4-stroke cycle, single acting diesel engine (National R4 AUM6) of 290 b.h.p. coupled through S.L.M. reverse-reduction gearing to a propeller having anti-clockwise rotation, viewed from aft to forward. Maximum engine revolutions are 486 per minute. Two lubricating oil pumps, one salt-water and one freshwater pump are incorporated in the main engine. The S.L.M. oil-operated marine gearing (Modern Wheel Drive, Ltd., type M2 WR, size 4) has a gear-ratio of 3·02–1. The blower is of Napier Turbo make, Type T.S. 100. The intermediate shaft runs in a self-lubricated plummer block and the stern tube has U.S. packing glands at the inboard and outboard ends. The propeller is of manganese-bronze, 4-bladed, left hand, of 7·75 ft. diam., and of 7·05 ft. mean pitch.

Fuel consumption at 125 shaft-r.p.m., giving an average (economical) speed of 8½ knots, is 1 ton in 24 h; the steaming range is 22 days, or just over 4000 miles.

Aspinal low-pressure indicators and alarms are fitted for lubricating-oil and cooling-water systems. A 'wrong way' alarm is also provided which operates if the propeller is moved contrary to telegraph orders.

All overboard discharges from engine-room and bilges are at the starboard side as far aft as practicable. By this arrangement the risk of contaminating water samples by ship wastes is reduced to a minimum, all water samples being collected over the port side in a forward position.

Auxiliary Propulsion. The slow speeds necessary in much scientific work cannot be provided by the main engine which has a lower limit of 3–4 knots. After much consideration of various possibilities, a separate low-speed drive was provided quite independent of the main engine. A 20 h.p. variable speed electric motor is geared to the propeller shaft through the main gear-box and so arranged that it cannot be engaged nor remain engaged except when the main engine is disengaged from the reverse-reduction gear. This arrangement works very well in calm weather, but a stronger electric motor would have given greatly improved performance in less favourable conditions.

Auxiliary Service Units. Two diesel-driven D.C. generators are installed—one 60 kW, 220 V driven by a National M4 A6 engine at 1000 r.p.m.; and one 35 kW, 220 V driven by a National M4 A3 engine at 850 r.p.m. Also driven off the M4 A6 engine is the V.S.G. power unit (V.S.G. type K, size 12) for operating the main (trawl) winch. This unit is activated through an S.L.M. clutch and reduction gear-box, ratio 1·54–1 (Modern Wheel Drive, size 1). A Reavall 11·5 cu.ft./min. 2-stage air compressor is driven off the M4 A3 engine. Aspinal alarms are fitted to both auxiliary diesels, as is also an 'Arkon' visual flow indicator for the freshwater systems. The main and auxiliary diesel engines all have freshwater circulation in a closed circuit with a common expansion tank. Heat exchangers are incorporated in the cooling systems.

Other auxiliary machinery in the engine-room comprises:

(1) A Russell Newbury diesel (7·5 b.h.p., 850 r.p.m.) driving a Reavall air compressor, 11·5 cu.ft./min., 2-stage; pressure 350 lb. sq.in.

(2) A Megator T 100 electrically driven general service pump.

(3) A Megator T 100 electrically driven bilge pump.

(4) Alfa Laval fuel and lubricating-oil centrifugal separators.

(5) A Megator M. 8 electrically driven domestic freshwater pump.

(6) A 3·45 single-phase, 50-cycle alternator. The alternator is controlled by an automatic carbon pile voltage regulator giving a stabilized voltage of 220 V. a.c. By hand regulation the unit can be made to provide any other voltage from 100 to 220, thus enabling continental and American types of a.c. mains apparatus to be used on board.

(7) A motor-driven V.S.G. 'A' end-unit for the aft hydrographic winch (boat deck).

The main engines and all auxiliaries are mounted on resilient pads of dexine to reduce vibration. All pipes leading from the engines are fitted with flexible pipe joints to prevent vibration transference from engines to pipes. The underside of the main deck over the engine-room, bulkheads and ship's sides are insulated against conduction of machinery noises to living quarters. Underneath the engine-room flooring, at the forward end, three echo-sounder oscillator tanks are fitted. Ventilation is provided by two Aerofoil dual duty fans situated in the funnel. One fan can be used for either supply or exhaust. Fuel oil is stored in three main bunker tanks having a total capacity of 30 tons.

Steering Gear. Brown's electro-hydraulic steering gear is fitted. The power unit in the steering flat consists of a motor-driven V.S.G. variable delivery pump (mark III, size I, type K). Normal telemotor control is provided from the bridge steering pedestal. The steering unit in the wheel-house remains operative even if the electric steering engine fails, a solenoid-operated valve in the oil system instituting automatic change-over to direct hydraulic control. If this also fails provision is made for emergency steering by hand control from the steering flat.

Windlass. The Reid electric windlass on the forecastle deck has a McClure motor of 11 b.h.p. at 800/1600 r.p.m.—half-hour rating.

Trawl Winch. The trawl winch is an Elliott and Garrood type VS 3–6 hydraulically operated three-drum model made to special order. The drive consists of one size 12, mark III V.S.G. 'A' end of 75 b.h.p. at 650 r.p.m. fitted with single acting auto-control; and one size 12, mark III V.S.G. 'B' end with speed 0–575 r.p.m. A total pull of $3\frac{1}{4}$ tons at an average hauling speed of 150 ft./min is provided.

Main Hydrographic Winch. This winch is hydraulically operated and incorporates type 'K' V.S.G. units, mark III, size III. The motor, V.S.G. 'A' end and starter are in the forward working compartment on the lower deck. The electric motor (by Laurence Scott) is of 25 h.p. at 720 r.p.m. The single drum carries 5000 m of 4 mm (diam.) wire. Average hauling speed is 200 m/min with a 300 lb. load. There is an automatic wire-spreader which can quickly and easily be disconnected if not required.

Aft Hydrographic Winch. This winch is also hydraulically operated and incorporates type 'K' V.S.G. units, mark III, size I. The motor, V.S.G. 'A' end and starter are all in the engine-room. The electric motor (by Laurence Scott) is of 75 h.p. at 720 r.p.m. There are two drums, one carrying 500 m of 4 mm (diam.) wire and the other carrying 300 m of 7 mm (diam.) wire. Average hauling speed is 200 ft./min with a 600 lb. load.

Heating System. A non-pressure boiler fitted with an 'electromatic' fully automatic oil burner supplies hot running water to bathrooms, living quarters and galley, and also provides central heating throughout the ship. The heating system can be shut off when not required while the hot-water supply is still in use.

Lighting. The vessel is fitted throughout with electric light and power of 220 V d.c. A shore connexion is provided for the purpose of drawing upon shore supply when berthed at a quay with machinery stopped. A rotary converter is incorporated in the lay-out and a change-over switch isolates the necessary circuits from the ship's internal supply system.

Secondary (emergency) lighting is provided from a set of 12 accumulators (see p. 398) housed in a special battery room, as is also the necessary current for bells, the Marconi 'Graphette' echo-sounder and alternative power for the radio-telephone.

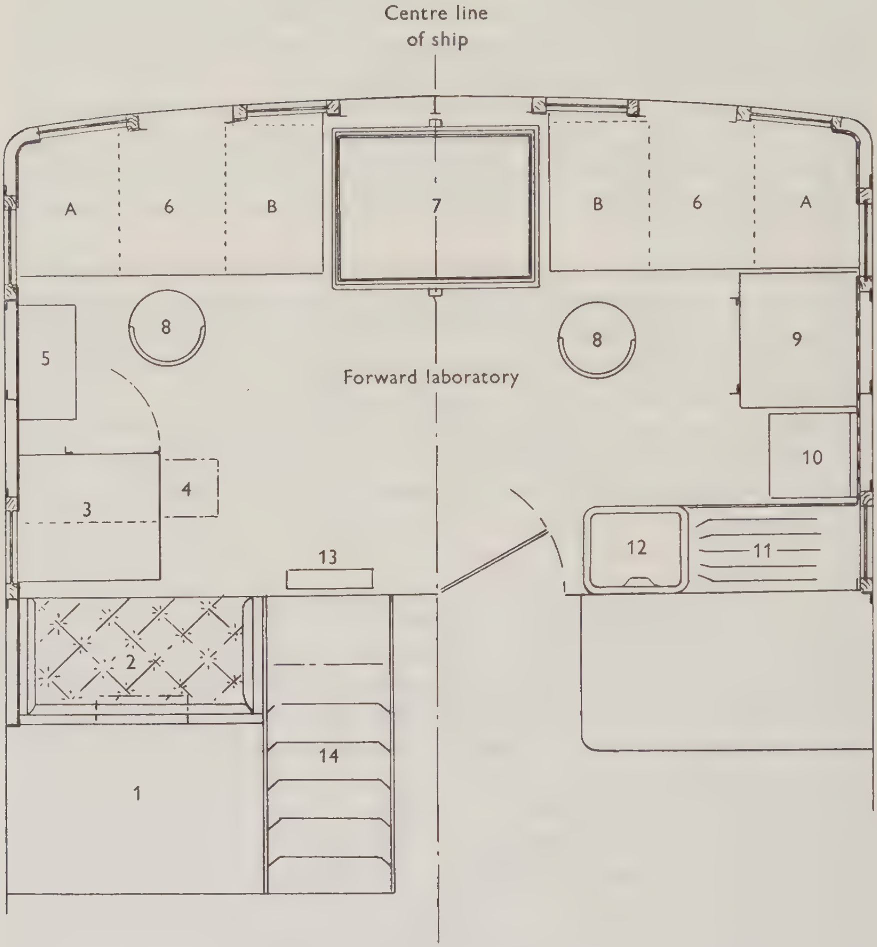
Scientific Arrangements and Equipment

There are two laboratories in the ship, both at main-deck level. They are accommodated in the deck-housing, one at the forward end immediately under the wheel-house and one at the after end under the boat deck.

The Forward Laboratory is designed as a general-purpose compartment. Its dimensions are 14 ft. 6 in. athwart ships, 8 ft. 6 in. fore and aft along the midships line and 10 ft. from deck to deck head. This unusual headroom became available when the wheel-house was raised 3 ft. above the general level of the boat deck. It will be of great benefit for carrying out physiological or other experiments in which a good hydrostatic 'head' of pressure is required, shelving being provided up to the full height of the bulkheads.

In a central position against the forward bulkhead a 'Discovery' type gimbal table is fitted, having surface dimensions of 3 ft. 4 in. by 2 ft. 6 in. On either side of this table, teak-topped laboratory benches extend to the full width of the laboratory; each is 5 ft. 3 in. long and approximately 2 ft. 6 in. in mean width.

Under each bench, in addition to ordinary drawers and cupboards, is a removable drawer unit fitted with cast aluminium-alloy drawer runners. Into these runners standard collecting-boxes are placed which then are used as ordinary drawers until the jars or bottles they contain are all filled. The full box is then lifted right out of its runner and sent below for storage in the racks provided in the working compartment (see p. 392) and a similar box of empty vessels brought up to replace it. By this arrangement the laboratory floor is


Centre line of ship
A
6
B
7
B
6
A
8
5
Forward laboratory
8
9
4
3
10
13
12
11
2
14
1

Text-fig. 3. Plan of forward laboratory.

kept completely clear of collecting-boxes which normally are awkward to accommodate and to handle in the restricted space available.

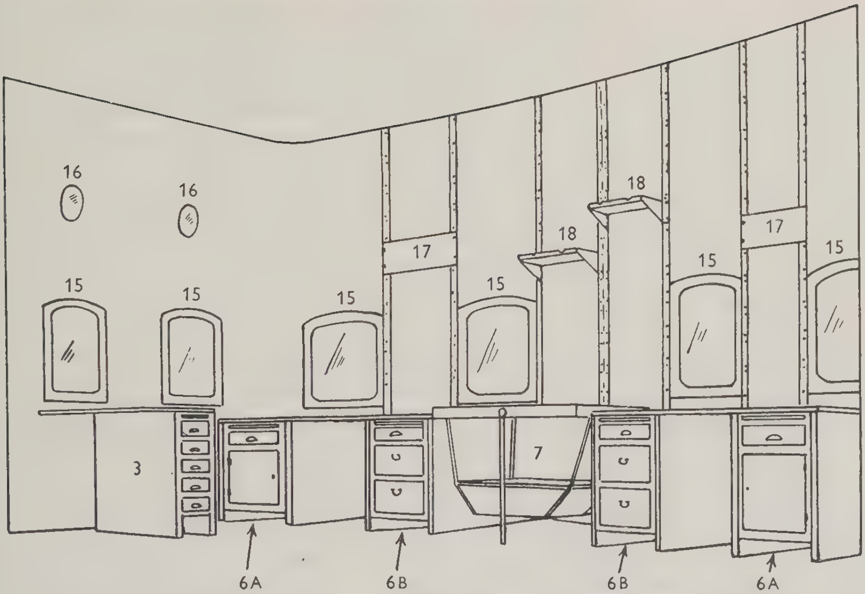
Four kinds of drawer units are provided which take different-sized boxes equipped with different collecting vessels.

Unit 'A' carries two boxes each holding 40 standard salinity bottles.

Unit 'B' takes two boxes each holding 6 full-sized breffits.

Unit 'C' takes two boxes each holding 15 half breffits.

Unit 'D' takes three boxes, one holding 15 half breffits, one holding 24 one-pound honey jars and one holding 28 half-pound honey jars.



Text-fig. 4. Perspective drawing of forward laboratory looking forward and to port.

Key to Text-figs. 3 *and* 4

1. Accumulator room.
2. Settee: terminal board over.
3. Bench with portable top: slide and drawers under.
4. Slide in pulled out position.
5. Aquarium tank with circulating sea water.
6. Laboratory bench with portable top: A, drawer and cupboards under; B, withdrawable drawer unit under. Knee-hole between A and B.
7. 'Discovery' type gimbal table.
8. Chairs.
9. } Hinged tables.
10. }
11. Draining board.
12. Sink.
13. Radiator.
14. Stairs down.
15. Windows fitted with armoured glass and storm screens.
16. Circular scuttles (openable).
17. Standard panels mounted on 19 in. standard post-office-type racks. Other standard racks are shown thus └ ┘ beside benches 3 and 9.
18. Special shelves mounted on the standard racks. Other specially fitted shelves shown in Pl. IV A. These can be moved or removed with the greatest of ease and others speedily fitted.

The spare space in each unit is taken up with a partitioned drawer and a pull-out writing board. Before the ship puts to sea the laboratory is provided with the drawer units appropriate to the type of work in hand. The spare units are stored in the working compartment near the racks holding the spare boxes.

Abaft the working bench, on the port side, a small glass-sided aquarium tank is fixed 3 ft. 9 in. above the deck and provided with circulating sea water. Its dimensions are 2 ft. × 1 ft. × 1 ft. high. A water-tight cover is fitted over the top of this tank to prevent spillage of water by the movement of the ship. The remainder of the space on the port side is occupied by a small bench (2 ft. 6 in. × 2 ft. 3 in.) with a pull-out writing board and drawers under. The after bulkhead on the port side is recessed to accommodate a small settee. In the back of the settee a hidden bench top is stored which can be pulled up and slid forward on the settee arms to provide additional bench space if the settee is not required.

On the starboard side of the after bulkhead is a laboratory sink provided with a hot and cold freshwater and a cold salt-water supply.

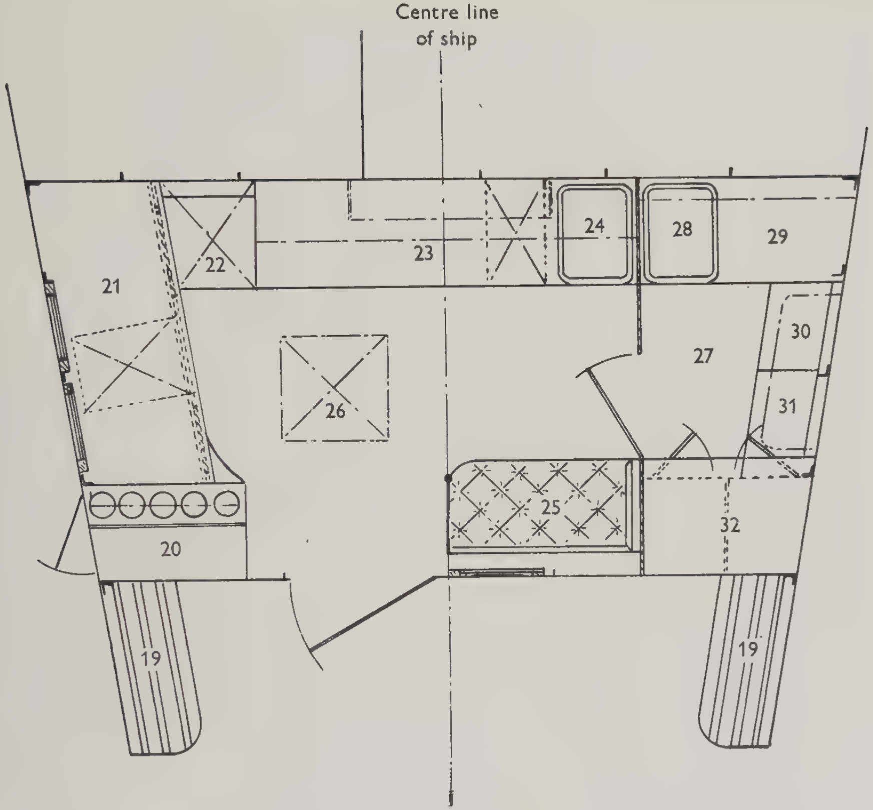
The starboard bulkhead is occupied by two hinged benches which can be folded down against the bulkhead to provide more floor space, if required.

Welded to the forward bulkhead of this laboratory are standard 19 in. post-office-type racks (as normally used for electronic assemblies) to which standard panels of wood or metal can be screwed at any height between bench and deck-head (Text-fig. 4 and Pl. IV B). On these panels any scientific assembly can be built up in a shore laboratory and screwed into position at any convenient place on the bulkhead racking and at any required height. To provide for apparatus assemblies that project on both sides of a panel, or which require access to both sides, other standard racks are provided extending from deck to deck-head, one on the port and one on the starboard side of the laboratory, in front of the side benches. These racks are so constructed that any scientific assembly erected on them can be swung outwards for easy access to any part of the assembly.

A number of panels have been made with special fitments to carry aspirator bottles, books and other standard apparatus (Pl. IV B). These can be accommodated in any position on the racking on the bulkhead, and can be moved to new positions at any time in a few minutes. Other special fittings can be erected on standard panels by any worker at any time, without visiting the ship, and with certainty of their being immediately accommodated on the standard racking on board whenever required.

Each working bench is provided with an additional (loose) whitewood top kept in position, when required, by a fiddle along the outer edge of the fixed top. These additional tops are provided so that any kind of apparatus of any shape or size can be screwed down upon them in any desired position without damage to the expensive permanent top underneath. The additional tops are cheap, expendable and easily renewed when unfit for further service. They can be stored away out of sight when not in use.

Abaft the main laboratory and abutting on its after bulkhead on the port side is a special 'accumulator room' carrying two banks of six accumulators, each bank producing 36 V and having a capacity of 100 amp/h. A selenium type



Text-fig. 5. Plan of aft laboratory and dark-room.

Key

19. Sparred seat.
20. Twine locker with access from outside deck; withdrawable unit under.
21. Bench, 3 ft. 3 in. high with drawers and cupboard under.
22. Bench, 2 ft. 6 in. high, hinged to bulkhead.
23. Laboratory bench, 2 ft. 6 in. high with shelf and bottle-racks over.
24. Sink.
25. Settee.
26. Escape hatch from crew's quarters (in floor).
27. Dark-room.
28. Dark-room sink.
29. Lead-covered bench: shelves over.
30. Hinged table: shelf over.
31. Hinged table.
32. Bench, 2 ft. 6 in. high with two equal cupboards under.

rectifier is employed for charging purposes, the rectifier being fed from the alternator or from shore supply, as required. The charging arrangements are so devised that one 'bank' can be on charge while the other is in service. The accumulators are connected, through the intervening bulkhead, with a terminal board in the laboratory in such a way that any voltage, in 2 V steps, may be drawn from it up to a total of 36 V.

A number of water-tight plugs and sockets with leads from the terminal board in the forward laboratory, are fitted in various parts of the ship—one each in the aft laboratory, on the flying bridge, in the forecastle store, in the working space, and on each side of the deck-housing, fore and aft. This does away with the need for temporary cables leading from the electrical supply points in the laboratory and strung inconveniently along the decks. A specially heavy cable connects the two laboratories. This is carried in a trunkway leading from one laboratory to the other, and along which additional temporary cables can be run at any time as required.

An Edwards RB 4 vacuum pump-compressor is fitted in the forward laboratory together with pressure and vacuum gauges. A service tap is provided at each bench and one on the oustide of the port bulkhead, available from the external deck.

Aft Laboratory. Access to this laboratory is by an outwardly-opening door from the after deck. On the port side is a working bench 3 ft. 3 in. high and 2 ft. 3 in. wide, with drawers and cupboards under. At one end a portable box/drawer unit (p. 396) is fitted. Along the forward bulkhead is another working bench 2 ft. 6 in. high with a sink and water supply at one end (starboard). Above is a specially designed rack for bottles, measures, books and other items. On the after bulkhead, to starboard of the entrance door, a small settee is fitted with a disappearing bench top stowed in the back like that of the settee in the forward laboratory (p. 398). The after bulkhead on the port side of the doorway is occupied by a twine locker with a door opening into it through the deck-house casing from the external deck. In this locker stowage is also provided for winchesters of formalin, spirit and other chemicals required from time to time to be readily available on deck.

Dark-room. Cut off from the aft laboratory by a wood partition and light-proof door is a small dark-room with sink, hot and cold fresh water, benches, cupboards, safelights and all necessary fittings.

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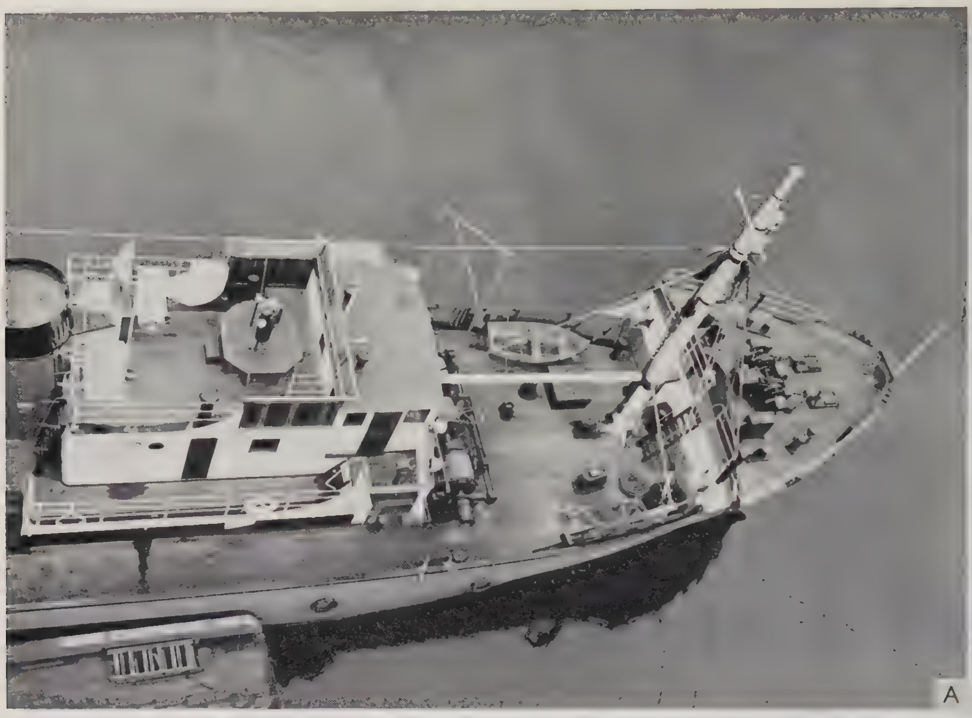
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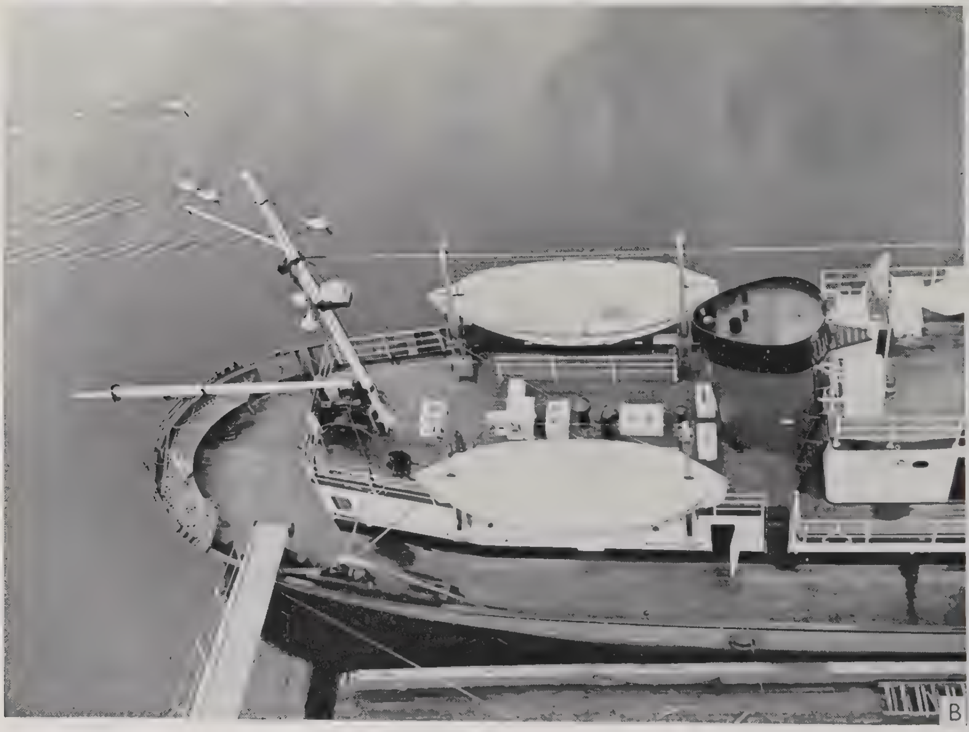
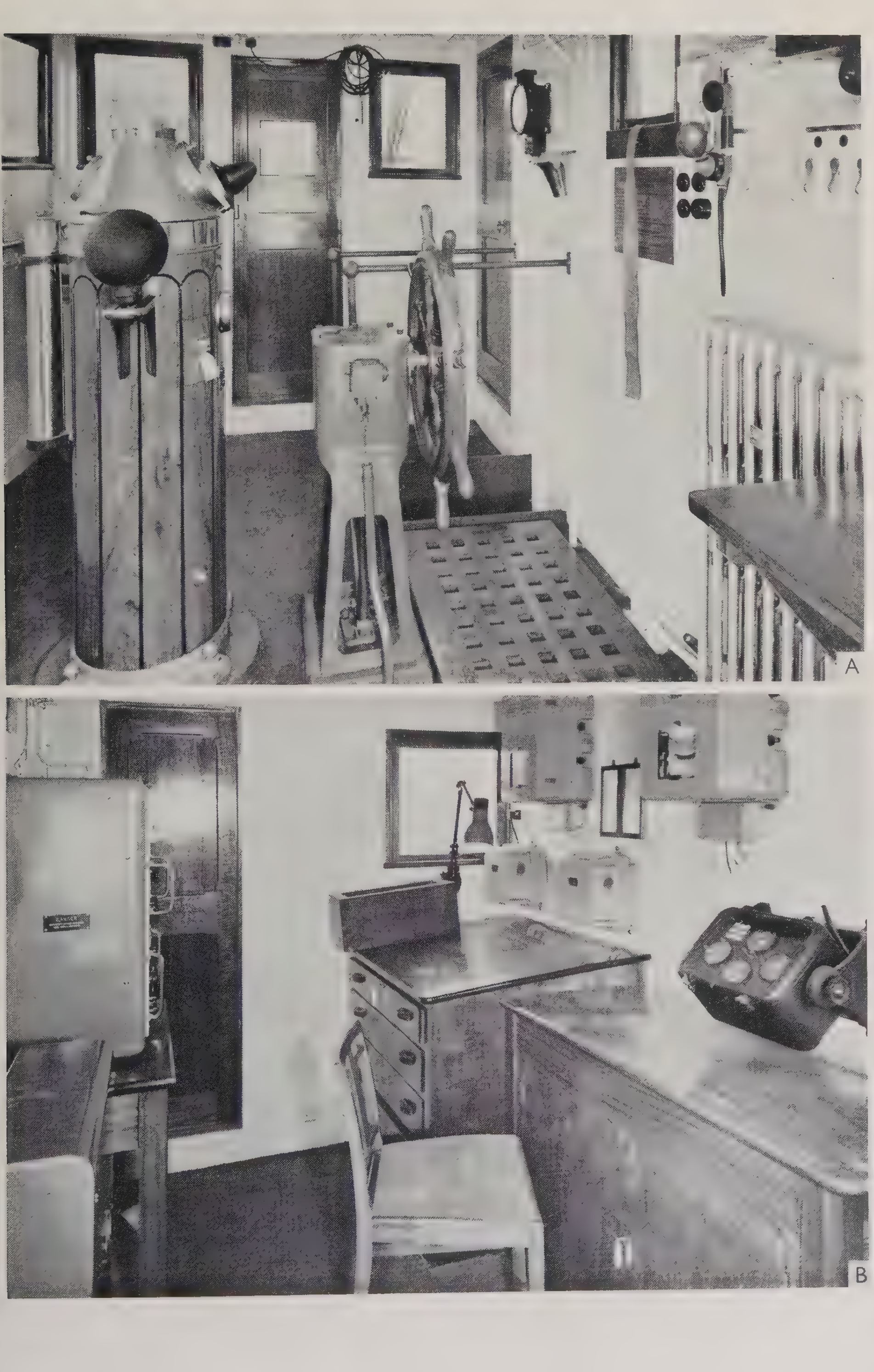

B


A
DANGER
B

(*Facing p.* 401)

EXPLANATION OF PLATES

PLATE I

Research Vessel *Sarsia* on arrival off Plymouth from the builders' yard—5 November 1953. Photo D. P. Wilson

PLATE II

A. General view of flying bridge, foredeck and forecastle deck.
B. General view of boat deck and after deck.

PLATE III

A. Wheel-house looking to starboard.
B. Chart-room, looking to port.

PLATE IV

A. Forward laboratory looking to port. Gimbal table in right foreground. Benches shown with removable tops in position.
B. Wardroom.

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ON THE BEHAVIOUR OF BARNACLES

I. THE RELATION OF CIRRAL AND OTHER ACTIVITIES TO TEMPERATURE

By A. J. Southward

From the Plymouth Laboratory and The Marine Biological Station, Port Erin

(Text-figs. 1–6)

INTRODUCTION

In previous papers (Southward, 1950, 1951; Southward & Crisp, 1952, 1954*a*, *b*: see also Kitching, 1950; Moore & Kitching, 1939) it has been shown that the geographical distribution of several common intertidal animals in Britain is related to the temperatures prevailing in the different regions. Species of generally southern distribution in Europe are commonest on, or restricted to, the south and west coasts of Britain, where temperatures are higher, while those species of essentially northern character are commoner in the north and east where the temperatures are lower. Where species of northern and southern distribution occur side by side in the same habitat, and are in competition, it seems important to know the range of temperatures over which processes such as feeding and respiration can be carried out, as well as the often narrower range of temperatures within which breeding can take place (cf. Orton, 1920). For example, the two common intertidal barnacles of the European coasts, *Chthamalus stellatus* (Poli) and *Balanus balanoides* (L.), occur side by side only in Britain and Northern France, where they reach the northern and southern limits respectively of their distributions. From the distributions it can be inferred that the vital activities of the two species are keyed to different temperature ranges. This implies that for each species there is an optimum range of temperature, above or below which the species becomes less efficient at feeding, respiring or reproducing. Where the two species occur side by side, there may be periods of the year when one of them is living outside its optimum range, and is at a disadvantage compared with the other species. Therefore, at any given place, the proportion of the annual temperature cycle which lies within the optimum range of temperature for a particular species' vital activities may then afford a measure or index of the relative success of the species there.

The most obvious activity of a barnacle, and one that may play an important part in both feeding and respiration, is the rhythmic beating of the cirri and the associated movements of the terga and scuta. It is likely that this activity reflects the general metabolic level of the barnacle. The frequency of beating of the cirri is simple to measure, but the experimental conditions necessary for uninterrupted beating are exacting. A barnacle is very sensitive to changes of temperature and illumination and to mechanical shocks; any of these stimuli may cause it to withdraw the cirri and close the terga and scuta. Specimens may also keep the terga and scuta closed in perfectly still water. If, however, a current of water is made to flow over the shell, sooner or later the valves part slightly, and a single cirral ramus may be cautiously put out; eventually rhythmic beating may start. Up to a certain current speed, which differs from species to species, the stronger the current, the less is the barnacle's response to other stimuli. A current of water thus assists the maintenance of regular beating. At high current speeds many species stop beating, and hold the cirri outstretched in the current for periods of up to several minutes (cf. Crisp, 1950), with occasional twisting movements from side to side. This reaction to strong currents can be termed the extension response. At even higher speeds, the terga and scuta may close again (closing response).

A further difficulty in assessing the cirral activity of barnacles is that at least two different types of beat may be shown by different specimens, or even the same specimen, under apparently identical conditions. The type of beat shown at times by all species and specimens can be termed the normal beat: the cirri are almost fully withdrawn into the shell between each extended phase, and the effect of increased temperature is to shorten the extended phase. Another type of beat has so far been observed only in *Balanus balanoides*, *B. crenatus* and *Elminius modestus*: in this beat, which can be termed the fast type, the closed phase is reduced or absent, and the extended phases follow so closely upon each other as sometimes to give the impression of a mere waving to and fro.

EXPERIMENTAL CONDITIONS

Barnacles were brought back to the laboratory attached to small pieces of rock, and were kept continuously immersed in running water. Some specimens had to be kept for several days before examination, and there was thus some risk of acclimatization to laboratory temperatures. In the later experiments specimens were examined within 48 h of collection, being meanwhile kept out of water in a cool damp place.

The cirral activity was observed in a shallow glass trough containing about 750 ml. of water, partially divided down the middle by a Perspex partition (Fig. 1).[1] Sea water from the aquarium supply was filtered through coarse silk mesh, passed through a large coil of glass tubing immersed in a large, thermostatically controlled water-bath, and supplied to one end of the trough through a flow-meter. Excess water overflowed at a constant level at the other end of the trough. The temperature of the running water was varied by heating or cooling the water bath, and its rate of flow controlled between 0 and 500 ml. per minute by a screw-clamp.

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[1] A slightly different apparatus was used for observations at Port Erin.

The barnacles were placed on the near side of the partition and viewed through a lens or dissecting microscope. On the far side of the trough was a paddle-wheel which could be driven, when required, by an electric motor controlled by a variable resistance. For some species the rate of flow of the water supplied through the heat exchange coil was sufficient to stimulate the cirri, but other species needed a stronger current, set up by means of the paddle-wheel, before regular beating could be obtained.

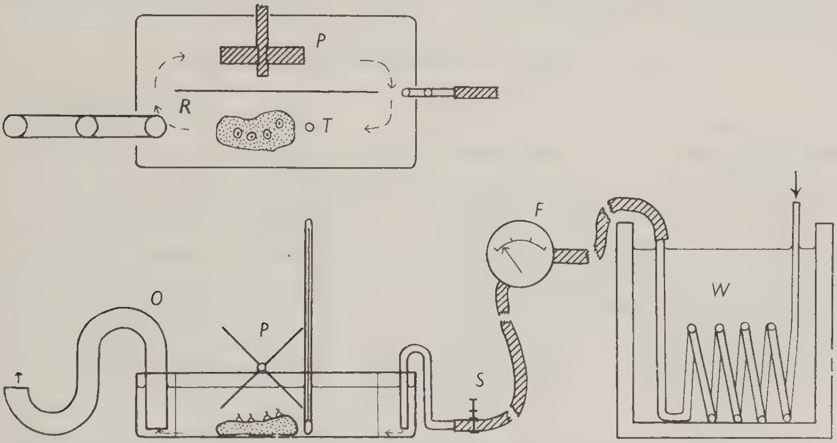

Fig. 1. Diagram of apparatus used for investigating the cirral activity of barnacles. Above: trough in plan; below: side view of whole apparatus. *F*, flowmeter; *O*, constant level outflow; *P*, paddle wheel; *R*, Perspex partition; *S*, screw-clamp on inlet tube; *T*, thermometer, scale about $\frac{1}{8}$th natural size; *W*, thermostatic water-bath and heat exchange coil, not on the same scale.

The frequency of beating was measured by recording with a stop-watch the time in seconds for ten complete openings and closings of the valves accompanied by protrusion of the cirri. The results were converted to the number of beats per 10 sec as this was the most convenient unit for later calculation of the standard deviation. All the results are quoted in these units. About ten specimens were tested at each temperature, and to ensure that so many were active it was necessary to have from two to five times this number in the trough (cf. Cole & Allison, 1937). The mean frequencies of beating given below therefore represent *the average activity of those barnacles active*, and not the activity of the whole sample. All temperatures are expressed in degrees Centigrade.

The water was heated or cooled slowly (about 5° per hour), and the temperature readings were taken close to the barnacles. The apparatus fluctuated in temperature by up to 0·5° during each experiment, but more rigorous control was not considered worth while, since in comparison with the intrinsic variation between individual barnacles the effects of such fluctuation were negligible. Results have therefore been grouped at the mean temperature of each experiment.

It will be noted that most species showed a large standard deviation, which at some temperatures approached the range of the observations. Each barnacle has its own frequency of beat which is only generally related to that of its fellows. A further probable cause of the great deviation from the mean may be the existence of several age-groups in the samples, for, as will be shown in a later paper, older barnacles beat more slowly than younger specimens. As far as possible the observations were confined to the predominant age-group in the population, excluding recently settled individuals. For most species this meant that specimens of approximately 1–2 years of age were investigated, but it was difficult to separate the age-groups perfectly by size and appearance (see Southward, 1955). In each species, also, the samples were taken from the optimum tide-level (Table I), and it is possible that different frequencies of beating would be shown by specimens from other tide-levels.

TABLE I. SPECIES OF BARNACLES INVESTIGATED

Species	Where collected	Tide-level	Speed of water-current in apparatus
Chthamalus stellatus (Poli)	Rum Bay, Plymouth	H.W.N.	5–10 cm/sec
Balanus balanoides (L.)	(*a*) Brixham, outer harbour	M.T.L.	0·1–0·2 cm/sec
	(*b*) Port Erin bay	M.T.L.	0·1–0·2 cm/sec
Balanus perforatus Bruguière	Wembury, nr. Plymouth	L.W.N.	0·5 cm/sec
Balanus crenatus Bruguière	Sutton harbour, Plymouth	L.W.N.	0·5 cm/sec, plus bursts of 5 cm/sec
Elminius modestus Darwin	Sutton harbour, and Henn Point, nr. Plymouth	L.W.N.	0·5 cm/sec, plus bursts of 5 cm/sec

RANGE AND FREQUENCY OF CIRRAL BEAT

The two common barnacles, *Chthamalus stellatus* and *Balanus balanoides*, showed quite different cirral behaviour even when compared at their optimum temperatures. The majority of individuals of both species remained closed in still water. But, while *B. balanoides* would set up rhythmic movements with a water current of only 0·1 cm/sec (≡ to a flow of 100 ml./min), *Chthamalus* sometimes needed currents up to 10 cm/sec (≡to 10,000 ml./min) to induce beating. At the latter speed *Balanus balanoides* tended to close the valves, while *Chthamalus*, especially the younger individuals, frequently showed the extension response.

Chthamalus stellatus

Even high current velocities would not induce *Chthamalus* to beat the cirri below 5°. Above this temperature the frequency of beating increased almost linearly with temperature to a maximum of 10 beats/10 sec at 30° (Table II; Fig. 2*c*). The frequency declined at 33° and fell off sharply at 36°, while all beating ceased at 37·5°.

As the normal temperature range experienced by this barnacle in Britain when beating the cirri (i.e. sea temperature) ranges from a mean of 6° in February to a mean of 16° in August, the species is quite clearly operating at the lower limit of its optimum range.[1] In winter, when the inshore water can drop below 6°, it may experience difficulty in feeding and respiring.

Balanus balanoides

The northern barnacle *B. balanoides* continued to beat the cirri down to 1·8°, and would probably remain active below this temperature. At temperatures above 2° the frequency of beating increased linearly to maxima of 6·2 (Port Erin) and 5·6 (Brixham) beats/10 sec at 17° (Table III; Fig. 2*a*, *b*). Above 17° the beating became more variable, although the mean frequency declined fairly steadily down to 30°. All beating ceased beyond 31–31·5°. The slight difference in mean frequency and standard deviation between the Port Erin and Brixham material was probably due to the inclusion of more age-groups in the former sample.

The widest range of monthly mean sea temperatures in Britain is 4–17°, found on the east coast. While *B. balanoides* is thus operating very near its upper optimum limit in Britain, there is no evidence that high temperatures have any detrimental effect on the species. It is, however, possible that the superiority of *Elminius modestus* in south-east England, is related to the latter species' tolerance of a wider range of temperatures than *Balanus balanoides*, as well as to its greater fecundity (Crisp & Chipperfield, 1948).

Balanus perforatus

Specimens of *B. perforatus* from Wembury were examined on two occasions, in November and June. The differences between the two sets of measurements are greater than was found with *B. balanoides* from different localities, but are in some part due also to a difference in age and size of the earlier sample.

It was not possible to induce this species to beat the cirri below 6°. Above 6° the mean frequency of beating increased fairly linearly to 25° (Table IV;

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[1] Note: the sea temperatures discussed on this page, and on subsequent pages are derived from the following sources: Admiralty, 1946; Air Ministry, 1949; Deutschen Seewarte, Hamburg, 1927; International Council, 1933; Lumby, 1935; Proudman, Lewis & Dennis, 1937.

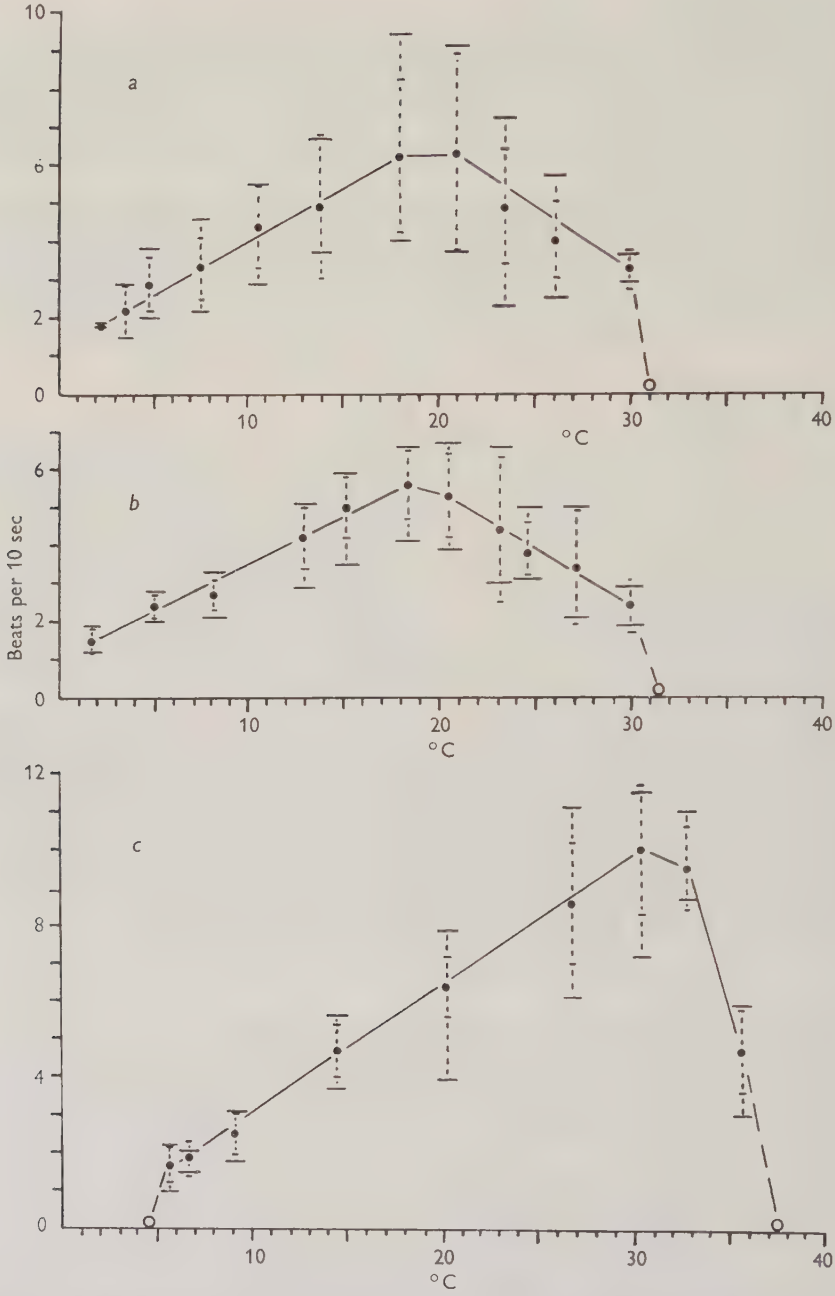

Fig. 2. The frequency and range of beating of the cirri of (*a*) *Balanus balanoides* from Port Erin, (*b*) *B. balanoides* from Brixham, and (*c*) *Chthamalus stellatus* from Plymouth. In this, and subsequent figures, the large open circles denote absence of cirral beat; small open circles refer to single observations only; the small dots mark the mean frequency of beat at each temperature; and the range of observations and the standard deviation at each temperature are shown by large and small cross-lines respectively on either side of the mean.

Fig. 3). Above 25° the beating varied considerably in both sets of measurements, generally tending to decline, although the secondary peaks of activity at 30 and 33° should be noted. Both series declined sharply at 35°, and beating ceased at 35·5–36°. The slightly higher mean frequencies shown by the June series may represent some acclimatization to summer conditions, but the variability at these temperatures prevents any certain correlation.

Like *Chthamalus*, *Balanus perforatus* is at the lower end of its optimum range in Britain, where it normally experiences mean sea temperatures of from 7 to 16°, and may also have difficulty in feeding and respiring during the winter.

Balanus crenatus

For *B. crenatus*, and for *Elminius* also, the most regular cirral movement was obtained by occasional stimulation with currents of 5 cm/sec in addition to the regular current of 0·5 cm/sec (obtained with water flow of 500 ml./min). The actual counts of frequency, however, were made with the regular flow only.

Balanus crenatus showed a comparatively rapid beat at low temperatures, and there was every sign that it would continue beating below the minimum temperature of 4° obtainable at the time. The mean frequency of beating increased more or less linearly with temperature from 4° to a maximum of over 10 beats/10 sec at 21° (Table V; Fig. 4). Above 21° the beating slowed down, and all cirral movement ceased at 25·5°. The great range of frequency of beat at the higher temperatures is noteworthy; to some extent it was due to the ability of some specimens to show the two types of beat already mentioned.

This barnacle is operating quite near its upper optimum limit in Britain, for temperatures above 20° may be experienced during the summer in the sheltered waters favoured by the species. Yet the same species is reported from tropical and subtropical localities by Darwin (1854) and Gruvel (1905). The cirral behaviour of the British specimens suggests that the species is here near the southern limit of its distribution, and supports previous doubts of the validity of the warm-water records (Pilsbry, 1916). Either the tropical records refer to another species, or *B. crenatus* shows a number of varieties adapted to different temperatures, such as are found in *Aurelia aurita* (Mayer, 1914). It is possibly significant in this connexion that in Britain the species is generally restricted to M.L.W.S. or below, and that above this level it is found only beneath stones or in the shade of thick growths of seaweed. These are all measures that would reduce the effect of the high temperatures that may be found on the shore in sunny weather.

Elminius modestus

Specimens of *E. modestus* were examined in February and in June. The February samples were obtained from two localities (see Table I), but the results have had to be combined to cover the whole range of temperature. Beating of the cirri could not be obtained below 2°; above this level the mean

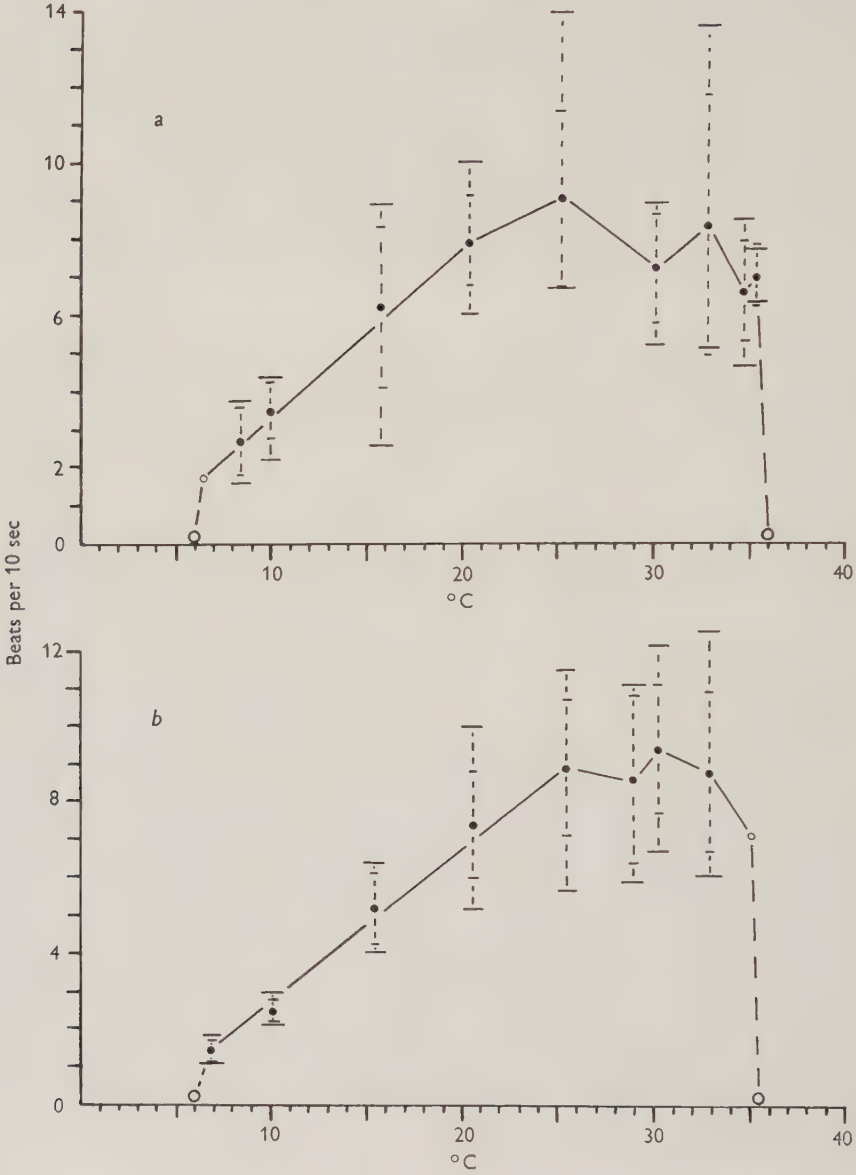

Fig. 3. The frequency and range of cirral beat of *Balanus perforatus*, (*a*) in November, (*b*) in June. For explanation of symbols refer to Fig. 2.

frequency of beating rose very rapidly to maxima of 22 beats/10 sec at 24° in February, and 18·5 beats/10 sec at 16·5° in June (Figs. 5 and 6). Beating then slowed down, declined sharply at 30° and ceased altogether at 33–35°. The difference in position of the maximum in each series is due to the two types of beat shown under apparently identical conditions. The June specimens showed a decline in the proportion of fast beat above 16°, while the February material showed a quite high proportion of fast beat up to 24°. Although the fast and normal beats can be distinguished by appearance, numerically their frequencies overlap and are difficult to separate. The wide deviation is probably also ascribable to the occurrence of the two types of beat.

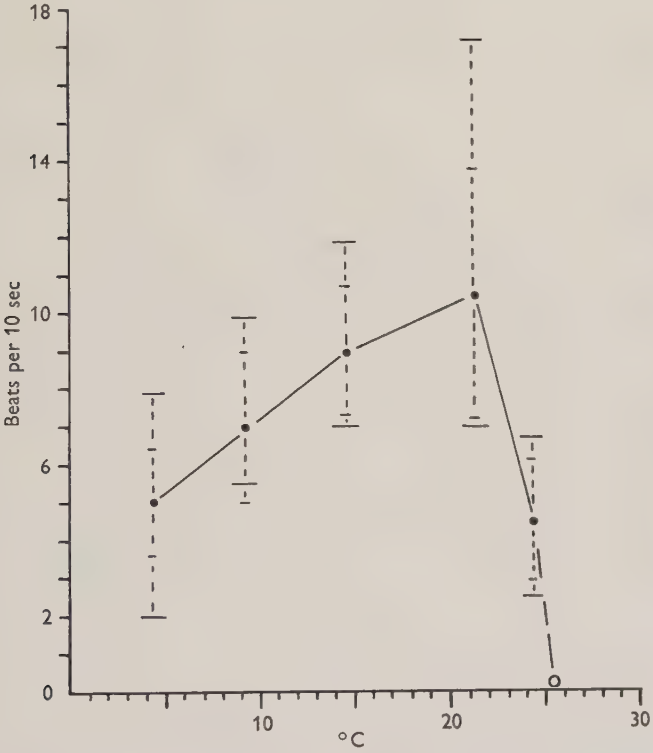

Fig. 4. The frequency and range of cirral beat of *Balanus crenatus*. For explanation of symbols refer to Fig. 2.

On the east coast of England *Elminius* may at times experience temperatures at the lower limit of its optimum range, and may sometimes be at a disadvantage compared with *Balanus balanoides*.

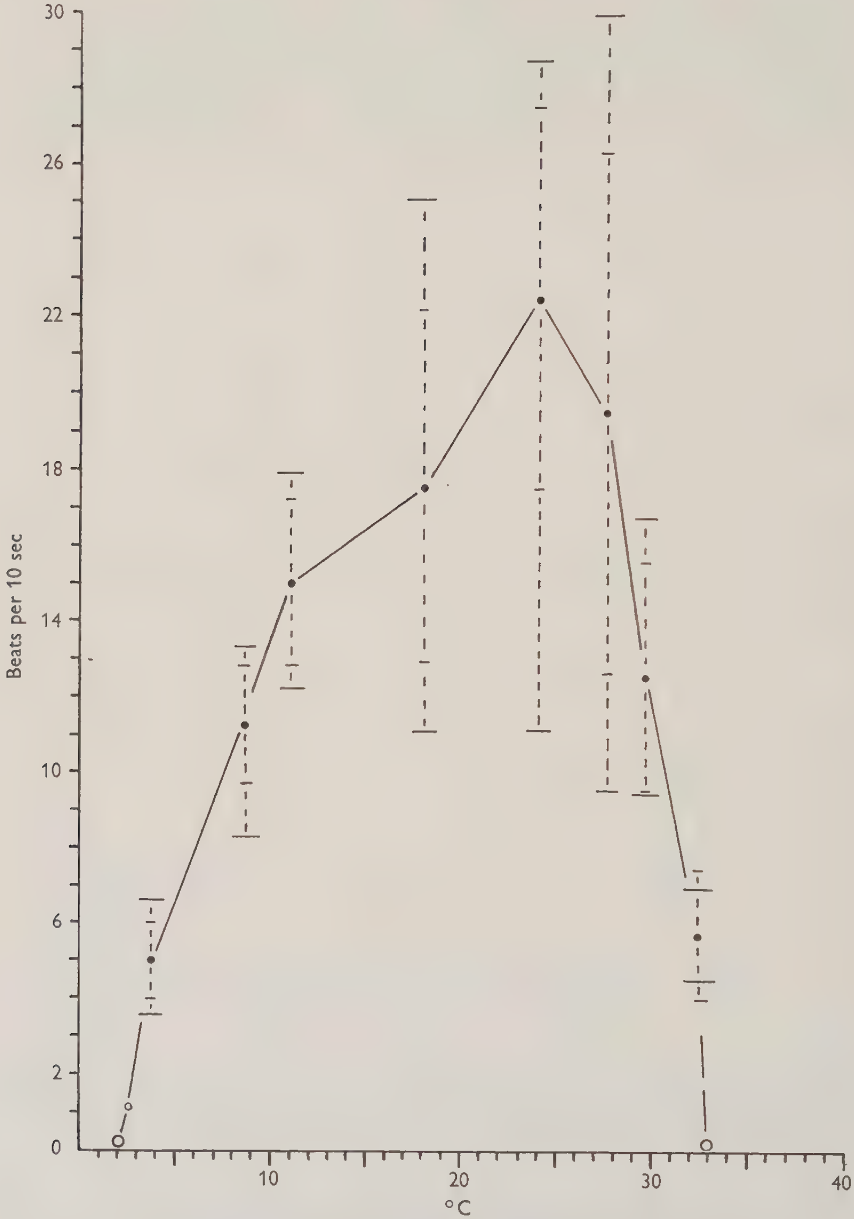

Fig. 5. The frequency and range of beat of *Elminius modestus* in February. For explanation of symbols refer to Fig. 2.

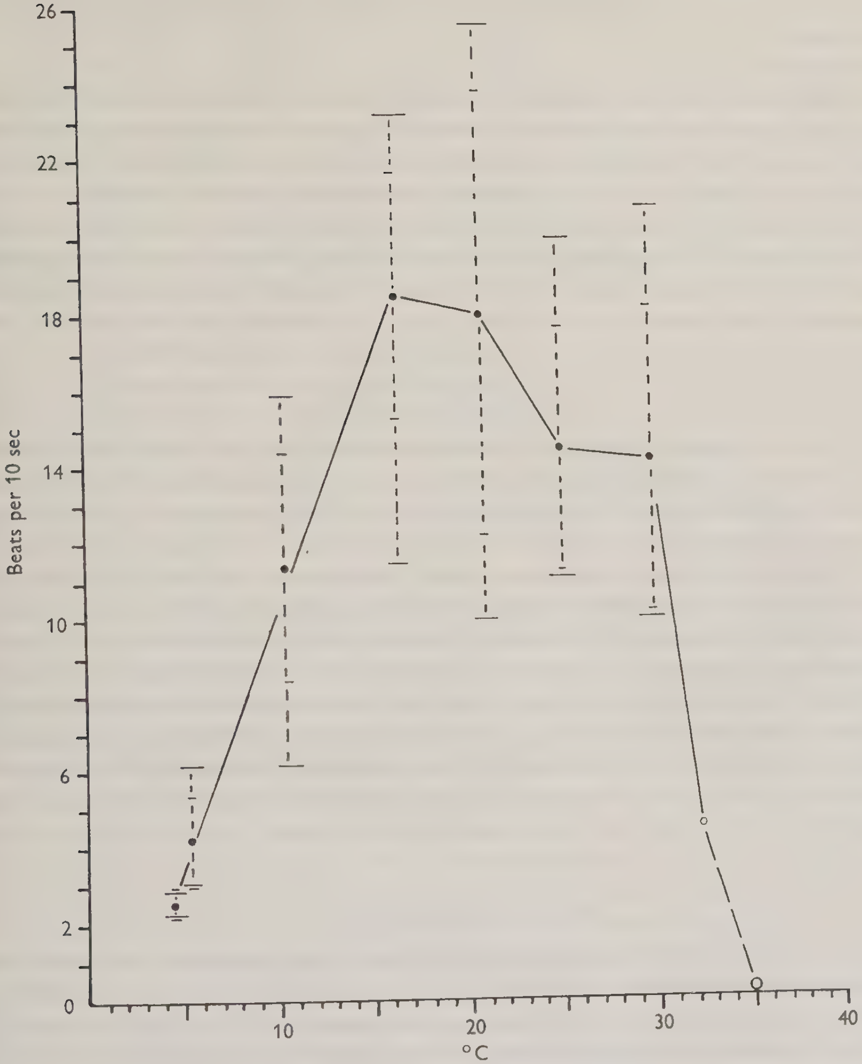

Fig. 6. The frequency and range of beat of *Elminius modestus* in June. For explanation of symbols refer to Fig. 2.

EXTREME RANGE OF ACTIVITY

A further indication of an animal's adaptation to the temperature range of its environment can sometimes be obtained by determining the upper and lower temperatures at which it loses irritability. When the point of heat coma or chill coma is reached it is reasonable to assume some temporary damage to other vital activities. These points were determined for the barnacles under investigation by slowly changing the temperature of the surrounding water until the normal reaction to touch, namely a closing of the terga and scuta, failed to occur.

Chthamalus, *Balanus balanoides*, *B. perforatus* and *Elminius* did not react to touch after an hour or more at 0°, but owing to difficulty in controlling temperatures at this level more precise information is not available. It is probable that some species can resist lower temperatures for shorter periods.

The points at which 50% of the samples showed heat coma were easier to investigate. The barnacles, still attached to small pieces of rock, were placed in 500 ml. of water, aerated with compressed air, and the temperature raised by heating the vessel in a small water-bath. The temperature was raised 1° per min, and direct observations with thermocouples showed that there was no lag greater than 0·1° between the barnacle and the surrounding medium. As might be expected from its range of cirral activity, *B. balanoides* was the first of the four species investigated to succumb to coma, at 35–37° in different experiments (Table VII). The remaining species followed in the order of their upper limit for cirral activity, *Chthamalus* being noteworthy in resisting coma above 40°.

These temperatures were experienced under water; on the shore they could be experienced only while exposed to the air, when the effect of desiccation would also be present. Towards high-water mark temperatures of up to 38° have been registered by thermocouples placed in the mantle cavity of barnacles during warm weather, although lower temperatures are more common over most of the British summer. It is interesting to note that of the two southern species *Chthamalus stellatus* and *Balanus perforatus*, the former, which occurs much higher up the shore and is therefore exposed longer to the heat of the sun out of water, has the higher point of heat coma.

REPRODUCTIVE ACTIVITY

Many barnacles appear to be cross-fertilizing hermaphrodites, and two of the species were observed copulating during the investigations of cirral activity. Both *Balanus crenatus* and *Elminius*, the former in February, the latter in June, continued to copulate down to 4° and up to 15°. The behaviour of the species was not affected in the least by the change in temperature, or by the temperature level within this range. Moreover, Dr Crisp informs me (personal communications) that he has observed copulation in *Balanus balanoides* at 17°, when the sea temperature in the normal habitat was about 8–10°. It appears probable, therefore, that the temperature limits for copulation, and possibly fertilization, are the same as those for cirral activity. Any limiting effect on reproduction by temperatures within these limits must operate on some other stage.

The process of copulation seems closely connected with cirral activity.

Both rhythmic beating of the cirri and penis extension are stimulated by water movement, and beating seems to be a necessary preliminary to penis extension. As observed in the course of the present work, the cirri first undergo a few rhythmic movements after stimulation, becoming more and more outstretched and stiffly-held as the penis unrolls; then both the unrolled penis and the cirri bend over towards the barnacle in the female phase and the penis is inserted into the mantle cavity of the latter. Usually the cirri are held flat, facing upwards, so that what is morphologically the ventral side of the animal is uppermost. As the penis is withdrawn and begins rolling up again, rhythmic beating recommences, although the valves may close after a few moments.

DISCUSSION

The Range of Activity

Some of the barnacles studied show a relation between the range of temperatures over which the cirri are active and the geographical distribution of the species. For example, the two southern species *Chthamalus stellatus* and *Balanus perforatus* were active from 5° and 6° to 38° and 35° respectively, and did not show any adverse effect of heat on the frequency of beating until nearly 30°. Neither occur in places where the mean sea temperature is lower than 5° or 6° in the winter months, but both venture into the tropics to places where the summer sea temperature may exceed 25°. The northern barnacle *B. balanoides*, on the other hand, was active from below 2° up to only 31° and the frequency of beating was affected by heat at 17°. This species ranges well within the arctic circle to places where subzero sea temperatures may be experienced in winter, but in Europe it does not occur where the monthly mean sea temperature exceeds 18° in the summer.

In addition to the higher minimum temperature for cirral activity in *Chthamalus* and *Balanus perforatus*, the rate of beating (and probably of feeding) of these species was markedly lower than that of *B. balanoides* at and below 10°. In Britain *B. perforatus* is absent from, and *Chthamalus* is scarce in, those areas where the mean sea temperature is much below 10° for several months in the year (i.e. those places where the accumulated temperature deficit below 10° is more than 10 month-degrees). The evidence suggests that where these barnacles occur together and are in competition, the temperature of the water is an important factor governing the success or otherwise of the species.

The activity of the above three species, then, is related to their distribution: the same cannot at present be said for *Balanus crenatus*. The British specimens of this species, judging from their behaviour in the laboratory, are more fitted to a distribution like that of *B. balanoides*, and the supposed tropical specimens must be either a different species or a physiologically adapted race. That the behaviour of a species may vary in different geographical localities seems at first sight affirmed by the differences shown between the British specimens of *B. balanoides* examined in the present work and some North American specimens examined by Cole, 1929 (see Table VIII). Although both the British and Maine barnacles were active below 2°, the former showed the first signs of the adverse effect of high temperature at 17°, while the Maine specimens were not affected until 23°. But, while the Maine barnacles ceased beating at 27° the British specimens carried on cirral activity to 31°. The range of monthly mean sea temperatures in Maine is about 4–17°, not different from that experienced in some parts of Britain. If the difference in range of activity of these two sets of barnacles is due to physiological adaptation, such adaptation cannot have any relation to present climatic conditions in either locality. The matter is dealt with further below.

It would be interesting to know the range of cirral activity of *Elminius modestus* in its original locality, Australasia. Although the British-bred specimens began to show the first signs of the adverse effect of heat between 20 and 25°, which agrees well with the highest monthly sea temperatures experienced in the native habitat (*ca.* 24°), they were able to carry on beating to 2°. This ability to withstand relatively low temperatures, while explaining the species success in establishing itself in eastern England and the Low countries (Hartog, 1953), does not appear to have any relation to the monthly mean sea temperatures experienced in Australasia in the winter (lowest, 7°). It is possible, therefore, that the species has acclimatized itself to European conditions.

The Rate of Activity

It is interesting that such similar animals should show so great a variation in the frequency of beating of the cirri. To some extent, as noted above, the differences are related to the geographical ranges of the species. A further part of the difference may well be an adaptation to colder or warmer temperatures than the animal experiences elsewhere in its distribution (cf. Fox, 1939), but until observations are made at other localities this cannot be determined.

Such an adaptation, however, would not seem to explain the differing frequencies of beat of the British and Maine specimens of *Balanus balanoides*. As already noted, present sea temperatures in Maine differ little from those in some parts of Britain: yet the frequency of beating of the Maine specimens was some two or three times faster, at a given temperature, than the British specimens examined in the present work (cf. Tables III and VIII). The differences in both range and frequency suggest that the two sets of barnacles are distinct varieties if not different species. It is intriguing to note that the whole pattern of behaviour of the *B. balanoides* from Maine is much more like that of the British *B. crenatus*.

In spite of the differences in frequency of beat, all the barnacles indigenous to Britain showed a temperature coefficient (Q_{10}) of between 2 and 3 up to their points of maximum activity. The immigrant *Elminius modestus*, however, showed a coefficient as high as 5 at some temperatures. This appears to have been due to a changeover from the normal to the fast beat with increasing temperature, i.e. a behaviour coefficient superimposed on the usual coefficient of a metabolism-coupled process. Unfortunately not enough specimens were examined at the time to show whether this was definitely the case. The phenomena of two different types of beat under identical conditions is dealt with in a later paper (Southward, 1955).

SUMMARY

The range of temperatures over which the cirri were active, and the frequency of beating of the cirri at different temperatures, were measured in five species of barnacles from the British intertidal zone. The range of temperatures at which copulation took place, and the extreme range of temperatures over which the animal remained irritable, were also investigated in some of the species.

The observed temperature range and frequencies of cirral beat differed in each species, but in the three barnacles common on the open coast were related to the geographical distribution of the particular species. The species of southern distribution, *Balanus perforatus* and *Chthamalus stellatus*, had a more rapid cirral beat at high temperatures, and continued active to much higher temperatures, than the northern species *Balanus balanoides*. Conversely, at low temperatures the latter had a greater frequency of cirral beat, and continued active to much lower temperatures, than the southern species.

The range and frequency of cirral beat of the British *B. balanoides* differed greatly from those observed by Cole (1929) for the species in North America. It is possible that the specimens belong to physiological races or even different species.

Of those barnacles more common intertidally in sheltered waters, *B. crenatus* showed an even narrower range of temperatures over which the cirri were active than did *B. balanoides*. It is suggested that the supposed tropical specimens of this species belong to a physiologically adapted race, or else previous doubts cast on the validity of the tropical records are well-founded.

The immigrant *Elminius modestus*, also more common in sheltered waters, showed a wider range of temperatures for cirral activity than *Balanus crenatus*. It was active at lower temperatures than were the native southern species, and at higher temperatures than were the native northern species, and over the whole of its range showed a much greater frequency of cirral beat than any of the native species examined. This behaviour, taken together with its great fecundity, could explain the success of *Elminius* in competition with the native barnacles.

Most of the investigations were carried out while I was holding a D.S.I.R. Senior Research Award at Plymouth; some observations at Port Erin were made while University of Liverpool Research Fellow there.

I am indebted to Dr D. J. Crisp for invaluable criticisms of the typescript, and for stimulating discussions on this work.

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TABLE II. FREQUENCY OF BEATING OF THE CIRRI, AS NUMBER OF BEATS PER 10 SEC, OF *CHTHAMALUS STELLATUS*

From H.W.N., Rum Bay, Plymouth, November 1953.

Temperature (° C)	Frequency		
	Mean	Range	S.D.
4·6		Nil	
5·7	1·660	1·0– 2·2	±0·50
6·8	1·90	1·5– 2·2	±0·538
9·2	2·470	1·8– 3·1	±0·544
14·5	4·70	3·7– 5·6	±0·653
20·3	6·355	3·9– 7·9	±0·815
26·9	8·633	6·1–11·1	±1·571
30·5	10·188	7·2–12·5	±1·747
32·9	9·475	8·7–11·0	±1·10
35·7	4·750	3·0– 5·9	±1·091
37·5		Nil	

TABLE III. FREQUENCY OF BEATING OF THE CIRRI, AS NUMBER OF BEATS PER 10 SEC, OF *BALANUS BALANOIDES*

(*a*) From M.T.L., Port Erin, Isle of Man, 15 to 22. iv. 53. (*b*) From M.T.L., Brixham, 26. i. to 3. ii. 54.

Temperature (° C)	Frequency		
	Mean	Range	S.D.
(*a*) 2·3	1·833	1·8–1·9	±0·065
3·6	2·20	1·5–2·9	±0·729
4·8	2·890	2·0–3·8	±0·717
7·5	3·350	2·2–4·6	±0·779
10·7	4·433	2·9–5·5	±1·089
13·9	4·944	3·7–6·7	±1·918
18·1	6·183	4·0–9·4	±2·069
21·0	6·30	3·7–9·1	±2·608
23·5	4·933	2·3–7·2	±1·563
26·1	4·043	2·5–5·7	±1·016
30·0	3·250	2·9–3·6	±0·495
31·0		Nil	
(*b*) 1·8	1·480	1·2–1·9	±0·254
5·0	2·360	2·0–2·8	±0·324
8·2	2·666	2·1–3·3	±0·427
13·0	4·181	2·9–5·1	±0·766
15·2	5·040	3·5–5·9	±0·820
18·4	5·550	4·1–6·6	±0·950
20·5	5·290	3·9–6·7	±1·162
23·1	4·40	3·0–6·6	±1·929
24·6	3·780	3·1–5·0	±0·834
27·2	3·433	2·1–5·0	±1·470
30·0	2·40	1·9–2·9	±0·70
31·5		Nil	

Table IV. Frequency of Beating of the Cirri, as Number of Beats per 10 sec, of *Balanus perforatus*

From L.W.N., Wembury: (*a*) 19. xi. 53; (*b*) 17. vi. 54.

Temperature (° C)	Frequency		
	Mean	Range	S.D.
(*a*) 6·0		Nil	
6·5	One only–1·7		
8·4	2·70	1·6– 3·8	±0·912
10·0	3·542	2·2– 4·4	±0·748
15·8	6·189	2·6– 8·9	±2·196
20·4	7·930	6·0–10·0	±1·237
25·2	9·030	6·7–13·9	±2·345
30·1	7·177	5·2– 8·9	±1·387
32·8	8·30	5·1–13·5	±3·427
34·7	6·625	4·6– 8·5	±1·307
35·4	7·014	6·3– 7·7	±0·835
36·0		Nil	
(*b*) 6·0		Nil	
6·9	1·350	1·1– 1·8	±0·309
10·1	2·450	2·1– 3·0	±0·301
15·5	5·150	4·1– 6·6	±0·892
20·6	7·425	5·2–10·0	±1·383
25·5	8·916	5·7–11·5	±1·798
29·0	8·611	6·0–11·1	±2·170
30·3	9·436	6·7–11·9	±1·664
33·2	8·850	6·0–12·5	±2·132
35·2	One only–7·1		
35·5		Nil	

Table V. Frequency of Beating of the Cirri, as Number of Beats per 10 sec, of *Balanus crenatus*

From M.L.W.N., Sutton Harbour, Plymouth, 16. iii. 54.

Temperature (° C)	Frequency		
	Mean	Range	S.D.
4·3	5·025	2·0– 6·9	±1·442
9·2	7·021	5·5– 9·9	±2·049
14·5	9·010	7·0–11·9	±1·729
21·3	10·530	7·0–17·2	±3·365
24·3	4·460	2·5– 6·7	±1·650
25·5		Nil	

TABLE VI. FREQUENCY OF BEATING OF THE CIRRI, AS NUMBER OF BEATS PER 10 SEC, OF *ELMINIUS MODESTUS*

(*a*) From M.L.W.N., Sutton Harbour and Hen Point, Plymouth 15–22. ii. 54. (*b*) From Hen Point only 22–24. vi. 54.

Temperature (° C)	Frequency		
	Mean	Range	S.D.
(*a*) 2·0		Nil	
2·5	One only–1·1		
3·8	5·037	3·6– 6·6	±0·973
8·7	11·166	8·3–13·3	±1·646
11·2	15·027	12·2–17·9	±2·247
18·2	17·518	11·1–25·0	±4·635
24·2	22·423	11·1–28·6	±5·020
27·8	19·431	9·5–29·8	±6·824
29·8	12·528	9·4–16·7	±3·005
32·5	5·70	4·5– 6·9	±1·697
33·0		Nil	
(*b*) 4·5	2·60	2·3– 2·9	±0·424
5·4	4·166	3·1– 6·2	±1·199
10·5	11·413	6·2–15·9	±2·993
16·5	18·472	11·5–23·2	±3·276
21·0	18·011	10·0–25·6	±5·789
25·1	14·446	11·1–20·0	±3·211
29·9	14·166	10·0–20·8	±3·960
32·4	One only–4·5		
35·0		Nil	

TABLE VII. TEMPERATURES AT WHICH IRRITABILITY WAS LOST BY 50% OF SAMPLE

Species of barnacle and tide-level	Chill coma*	Heat coma†
Chthamalus stellatus from M.H.W.N.	0°	43°
Balanus balanoides from M.T.L.	0°	35 to 37°
B. perforatus from M.L.W.N.	0°	38 to 40°
Elminius modestus from M.L.W.N.	0°	36 to 38°

* After more than an hour; owing to the difficulty in controlling temperatures at this level shorter periods could not be observed; it is possible that some species can withstand lower temperatures.

† Instantaneous, at a rate of heating of 1° per min; direct measurement with thermocouples in the mantle cavity showed no lag greater than 0·1° between the water and the barnacle.

TABLE VIII. FREQUENCY OF BEATING OF THE CIRRI, AS NUMBER OF BEATS PER 10 SEC, OF SPECIMENS OF *BALANUS BALANOIDES* AT MT DESERT ISLAND, MAINE, SUMMER 1929

Recalculated from Cole, 1929.*

Temperature (° C)		Range of beat
	Some activity below 2°	
5		5·6– 7·9
9		9·3–12·0
13		12·9–17·8
17		15·1–20·9
21		16·6–23·4
26		19·0–24·5
27	beating ceased	

* Cole did not give any tables: these figures are derived from his graphs which were fitted to the Arrhenius equation. The abscissae are given as 'log 100 × rate' which has been assumed, on the basis of a later paper (Cole, 1932) in which some raw data is given for the same barnacles, to mean really the log of the reciprocal of the time in seconds for ten beats multiplied by a hundred.

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ON THE BEHAVIOUR OF BARNACLES

II. THE INFLUENCE OF HABITAT AND TIDE-LEVEL ON CIRRAL ACTIVITY

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During a study of the relation between temperature and cirral activity of barnacles, reported in Part I of this series (Southward, 1955), specimens for investigation were collected from those habitats and tide-levels where the particular species was most abundant. However, it is possible that some variation in behaviour may arise from local environmental factors such as tide-level and degree of exposure to wave action, and experiments were carried out to test this possibility.

The experimental conditions under which the barnacles were observed, and the methods of measuring cirral activity, were similar to those described in Part I. In all experiments the material was dealt with within 24 h of collection from the shore. Only one or two temperature levels were used to obtain temperature-frequency relations, as this was sufficient to enable a comparison of behaviour to be made between samples from different localities or tide-levels. Most frequently the temperature of the laboratory sea water supply was used since it required no regulation and remained at uniform temperatures (within 0·5 to 1·0°) for long periods.

The investigations were carried out during the first half of 1954, during the tenure of a D.S.I.R. Senior Research Award. I am grateful to Dr D. J. Crisp for help and advice in this work.

INVESTIGATIONS AT PLYMOUTH

Chthamalus stellatus

The work at Plymouth was confined largely to the commonest barnacle in the neighbourhood, *C. stellatus*. Specimens were first taken from two tide-levels at Tinside, below the laboratory, and examined at two temperatures. At both temperatures the two age-groups present in the sample from low water showed a 40–50% greater frequency of cirral beat than that of specimens of corresponding age from high water (Table I). This difference, when tested by the *t* distribution, was found to be significant in comparison with the individual variation (P less than 0·1% that the samples from the two tide-levels came from the same population).

Subsequently, however, specimens were taken from high and low tide levels at Wembury, and similarly compared. In this case there was very little difference in the frequency of cirral beat, and the slight differences that were observed were not statistically significant (Table II). The experiments were therefore repeated on some more specimens from Tinside, and simultaneously observations were made on barnacles from Rum Bay, a nearby site in Plymouth Sound (Table III). Again the specimens from L.W.N. at Tinside had a significantly faster cirral beat than those from high water, while there was no significant difference between the two sets of barnacles from Rum Bay.

TABLE I. FREQUENCY OF BEATING OF THE CIRRI, AS BEATS/10 SEC, OF SPECIMENS OF *CHTHAMALUS STELLATUS*

From two different tide-levels at Tinside, Plymouth, 15. xii. 53.

Temperature (° C)	Year-group	Tide-level	No. in sample	Frequency			
				Mean	Range	S.D.	*t*
13·8	1+	M.H.W.S.	6	3·516	2·2–4·5	0·893	3·33
		M.L.W.N.	12	5·466	3·8–8·4	1·205	
	2+	M.H.W.S.	9	3·188	2·3–3·7	0·459	6·36
		M.L.W.N.	10	5·810	4·1–8·1	1·184	
20·4	1+	M.H.W.S.	8	5·962	4·5–7·4	0·976	7·65
		M.L.W.N.	20	9·725	7·8–12·1	1·240	
	2+	M.H.W.S.	9	4·822	3·7–6·1	0·882	4·78
		M.L.W.N.	12	8·350	6·4–10·2	1·389	

TABLE II. THE FREQUENCY OF BEATING OF THE CIRRI, AS BEATS/10 SEC, OF SPECIMENS OF *CHTHAMALUS STELLATUS*, APPROXIMATELY 1½ YEARS OLD

From two different tide-levels at Wembury, near Plymouth.

Date	Temperature (° C)	Tide-level	No. in sample	Frequency			
				Mean	Range	S.D.	*t*
22. i. 54	20·4	M.H.W.S.	9	5·220	3·8–6·6	0·788	1·07
		M.L.W.N.	10	5·630	4·3–7·2	0·869	
19. ii. 54	19·6	M.H.W.S.	7	5·185	4·2–7·3	1·083	1·21
		M.L.W.N.	8	5·837	4·4–7·1	0·985	

TABLE III. THE FREQUENCY OF BEATING OF THE CIRRI, AS BEATS/10 SEC, OF SPECIMENS OF *CHTHAMALUS STELLATUS*, APPROXIMATELY 1 YEAR OLD

From two different tide-levels at two places in Plymouth Sound, 1–2. vi. 54, temperature 14·9° C.

Place	Tide-level	No. in sample	Frequency			
			Mean	Range	S.D.	*t*
Tinside	M.H.W.S.	9	6·355	5·3–7·7	0·705	3·03
	M.L.W.N.	10	7·780	6·3–9·5	1·014	
Rum Bay	M.H.W.S.	17	6·576	4·9–8·9	1·368	0·45
	M.L.W.N.	10	6·370	5·6–7·2	0·566	

The matter rests there at the moment. It can only be suggested that the *Chthamalus* at low water at Tinside are anomalous compared with those from all other levels and localities, but the reason for this anomaly is not clear.

Elminius modestus

Further tests were made on another barnacle, *E. modestus*, which at Plymouth is common only in the estuaries. Specimens under 1 year old showed a significant difference in cirral beat between individuals from different tide-levels (Table IV). Those from low water had a mean frequency of beat some 20% greater than those living at high water. The older age groups of this species were not tested, as the older specimens among the high water sample could not be induced to beat regularly under laboratory conditions.

TABLE IV. THE FREQUENCY OF BEATING OF THE CIRRI, AS BEATS/10 SEC, OF SPECIMENS OF *ELMINIUS MODESTUS*, UNDER 1 YEAR OLD

From two different tide-levels at Hen Point, near Plymouth, 22. ii. 54, temperature 12·4° C.

Tide-level	No. in sample	Frequency			
		Mean	Range	S.D.	*t*
M.H.W.N.	6	15·350	13·3–16·7	1·126	3·10
M.L.W.N.	9	18·200	15·6–21·8	1·857	

TABLE V. OBSERVATIONS ON *CHTHAMALUS STELLATUS* FROM M.H.W.N., HEN POINT (TAMAR ESTUARY), 23. vi. 54, TEMPERATURE 16·6°

Age group, in years	Percentage of total sample showing rhythmic beating of cirri	Mean frequency of beating, as beats/10 sec
1	50	8·066
2	30	6·433
3 and over	12	5·200

Effect of Age

The difficulty of obtaining regular beating from the older barnacles was frequently encountered during the work described in Part I. The irregular behaviour of the older barnacles seems to be linked with their generally lower mean frequency of cirral beat (see Table I). Some comparative observations showed a decrease, with increasing age, of both the frequency of beat and the proportions of the sample regularly beating (Table V). The two phenomena are probably characteristic of the ageing process in barnacles.

Unfortunately it is not possible to determine critically the effect of age on cirral activity without more exact information of the age of the barnacles examined. The age-groups given here were separated only by estimation, based on experience of the barnacle in question, its size at settlement and its known rate of growth. Although this was sufficient to show that the younger barnacles have more lasting and frequent cirral activity, further investigation with barnacles of definite age is clearly desirable.

The work at Plymouth indicated that habitat variations might have some influence on the cirral activity of barnacles. It was therefore arranged to continue the work in the Anglesey area (from the Marine Biology Station at Menai Bridge), where a greater variety of habitats were within easy reach, and where the other common British intertidal barnacle, *Balanus balanoides*, was plentiful.

Investigations at Menai Bridge

The experimental conditions at Menai Bridge were similar to those at Plymouth. The water currents used in the apparatus, in addition to a regular flow of water through it, were set up by a jet of compressed air instead of a paddle wheel.

Table VI. The Frequency of Beating of the Cirri, as Beats/10 sec, of two Species of Barnacles

From two different tide-levels at Criccieth, 26. iv. 54, temperature 10·4° C.

Species	Approximate age (years)	Tide-level	No.	Frequency			
				Mean	Range	S.D.	*t*
Chthamalus stellatus	1½	M.H.W.N.	10	2·970	2·1–4·2	0·602	0·72
		M.L.W.N.	10	2·950	1·9–3·7	0·632	
Balanus balanoides	1	M.H.W.N.	20	3·155	2·1–4·1	0·584	12·53
		M.L.W.N.	20	4·705	3·9–6·0	0·664	

Effect of Tide-Level

Specimens of *Chthamalus stellatus* and *Balanus balanoides* were collected from two tide-levels at the north end of Cardigan Bay, and examined the same day. As can be seen from Table VI, there was no significant difference in the frequency of beat of the *Chthamalus* from the two tide-levels. In the case of *Balanus balanoides*, however, the specimens from low water had a markedly faster cirral beat than those from higher up the shore. This difference in behaviour of two species living side by side under identical conditions is surprising, in view of the great similarity, at the temperature used, of the frequency of beat of the high water individuals of both barnacles. It is interesting to note that, again at this temperature, the low water *B. balanoides* would probably be more successful in obtaining food than the *Chthamalus* at the same level.

Further experiments on the same barnacles during subsequent days showed a change in behaviour. After the specimens had been kept for 24 h in running sea water from the laboratory supply the former difference between the high and low water *Balanus balanoides* disappeared, the mean frequencies being, at 18°: high water, 7·45; low water, 7·72. The corresponding frequencies for *Chthamalus* at 17° were: high water, 5·17; low water, 4·85. The similarity between the two sets of *Balanus balanoides* was found again the following day, when the mean frequencies at 10·4° were: high water, 5·62; low water, 5·56, Comparison with Table VI shows that both the high-water and the low-water samples of *B. balanoides* had increased their frequency of beat after only 3 days in the laboratory, but that the increase was greater among the individuals from the higher tide-level.

These experiments serve to show the extent of acclimatization that may occur. It is not known whether the change in the frequency of beating was a reaction to some quality of the laboratory water (temperature, salinity, pH, silt, etc.), or merely to the continued immersion. Cole (1929, 1932) found no change in the frequency of beat of continuously immersed *B. balanoides* at Mt Desert Island, Maine, but, as noted in Part I, there are important differences in the behaviour of the British and American specimens of this species.

Effect of Habitat

Some preliminary experiments suggested that the fast type of beat (see Part I) was more commonly shown by individuals from sheltered habitats, than by those living on the open coast. A full investigation was therefore undertaken with *B. balanoides*, by collecting a series of samples from four different habitats on the same day. This was possible since the variation in the time of low water around Anglesey permits several localities to be visited on one tide. To avoid acclimatization changes the samples were kept out of water at the laboratory, in a cool place, and examined within 24 h. The measurements of the cirral beat were made at approximately the time of high water the following day, to minimize any inherent tidal rhythm of the animals. Before counting the frequency of beating each sample of barnacles was allowed 30 min in water to eject the air that is often present in the mantle cavity, and to settle down to regular beating. The numbers showing fast beat, normal beat, and those closed or irregular were then noted (Table VII).

It was found that there was invariably a larger proportion of closed or non-beating individuals among the specimens from high water compared with those from lower down on the shore. Furthermore, a much lower percentage of rhythmically beating individuals occurred among the barnacles from the open coast site exposed to wave action than was found at the three sheltered localities. This was evident in samples from both tide-levels. This difference may be habitual, for those barnacles living at high water may be used to rhythmic beating only for short periods when covered by the tide. Individuals from a wave beaten place may also be unused to beating continuously for a different reason. If the continuous water movement resulting from wave action brought an abundant supply of food and oxygen to the barnacles, only limited periods of cirral activity might be necessary for normal growth and respiration.

TABLE VII. THE FREQUENCY OF BEATING OF THE CIRRI, AS BEATS/10 SEC, AND THE PROPORTION OF INDIVIDUALS ACTIVE, OF SAMPLES OF APPROXIMATELY 1 YEAR OLD *BALANUS BALANOIDES*

From different tide-levels and habitats, Anglesey, 30. iv. 54

Locality, and type of habitat	Temperature °C	Tide-level	Fast beat					Ordinary beat					% closed or not beating
			% of sample	No. examined	Frequency			% of sample	No. examined	Frequency			
					Mean	Range	S.D.			Mean	Range	S.D.	
Aberffraw, open coast exposed to wave-action	13·0	M.H.W.N.	0	—	—	—	—	55	8	3·975	2·6–5·9	1·149	45
		M.L.W.N.	0	—	—	—	—	64	10	5·010	3·9–6·9	0·898	36
Aberffraw, open coast sheltered from wave-action	12·2	M.H.W.N.	0	—	—	—	—	81	11	3·127	2·5–4·1	0·735	19
		M.L.W.N.	2	—	One only 13·3			87	12	5·950	3·1–9·8	2·153	11
Menai straits, exposed to currents	12·6	M.H.W.N.	3	—	One only 10·2			82	10	2·930	1·9–4·4	0·707	15
		M.L.W.N.	3	—	One only 10·8			86	10	6·010	4·2–7·4	1·275	11
Menai straits, sheltered from currents	11·6	M.H.W.N.	68	10	15·560	13·9–16·7	0·938	12	3	9·400	7·7–10·5	1·493	20
		M.L.W.N.	0	—	—	—	—	89	10 .	5·640	4·5–8·3	1·153	11

Note: the number examined is not the number on which the percentages are based, except in one case.

Turning to the mean frequency of the normal cirral beat shown by these samples (Table VII, col. 11) it can be seen that, except for the specimens from the sheltered site without currents, the low-water samples had a mean 30–100% greater than that of the high-water individuals. The least difference was shown by the barnacles from the wave beaten locality at Aberffraw, and the greatest by those from the site in the Menai Straits exposed to currents.

The fast type of beat was not shown by any individuals from the wave-beaten site, and only one fast-beating specimen occurred in the sample from the sheltered open coast site, and two among those from the site with currents. However, among those individuals from the site without wave action or currents, a remarkably high proportion of fast-beating specimens was found in the high-water group. At low water in the same place not a single fast-beating specimen was obtained. On ecological grounds it seems possible that the fast type of beat could, in this case, be an adaptation to life in comparatively still water, a replacement of the natural movement of water over the cirri found in the other habitats. It might only be found regularly among barnacles from high water, since these would have the greatest need of such an adaptation on account of the short time available for feeding and respiratory exchange.

Effect of Oxygen Content

If respiratory need were the driving factor in stimulating the fast type of cirral beat, it should be possible to investigate the relation by varying the oxygen content of the medium, and recording the effect on the type and frequency of beat. Specimens of *B. balanoides* together with *Chthamalus stellatus* for comparison were observed in a small dish holding about 200 ml. water with a lid ground on and sealed with vaseline. Gases were led in through calibrated flowmeters of the manometer type, and bubbled through the water in a manner that caused currents to be set up. Observations were made at regular intervals, the flow of gas being turned off momentarily to arrest the water currents. The temperature was kept fairly constant by immersing the dish in a trough of running tap water.

The effects of lowering the oxygen content by bubbling nitrogen through the water, and of increasing the oxygen content by bubbling that gas through the water, are given in Table VIII, for individual specimens of *Balanus balanoides* and *Chthamalus*. It should be noted that, as the supply of nitrogen and oxygen did not contain any carbon dioxide, the latter may have been driven off from the water. The loss of some of the carbon dioxide, and the resulting decrease in the hydrogen-ion concentration, may have had some influence on the behaviour of the barnacles. The results can only be accepted as indications of responses to variations in the oxygen content on the assumption that the possible changes of carbon dioxide content and pH had a negligible effect. Previous work (Roaf, 1912; Fox & Johnson, 1934) suggests that the effects of a decrease in the carbon dioxide content and hydrogen-ion concentration are indeed negligible, though an *increase* may influence the cirral beat.

From the table it can be seen that lowering the oxygen content of the water caused a reduction in the cirral activity of both fast and normally beating specimens of *Balanus balanoides*, and had a similar effect on *Chthamalus*. In each case, the first response was a slowing down of the frequency of beating, followed by a cessation of beating altogether. At the end of the experiments practically all specimens had the terga and scuta slightly open, often with the cirri protruding. This reaction can be termed the gaping response, and probably indicates acute respiratory difficulties (cf. Fox & Johnson, 1934). It will be noted that before the fast-beating specimens became inactive they changed over to the normal type and frequency of beat. From this it might be inferred that the fast type of beat is not related to respiratory need, but it must be remembered that the fast beat in itself may utilize more oxygen than the

TABLE VIII. THE EFFECT OF VARYING THE OXYGEN CONTENT OF THE WATER ON THE FREQUENCY AND TYPE OF CIRRAL BEAT OF INDIVIDUAL BARNACLES, 28–29 iv. 54

A. Oxygen decreased by passing nitrogen through the water at 6 ml./min; temperature 13° C.

Time in min since start of N_2 bubbling	Frequency of individual specimens*											
	Balanus balanoides								*Chthamalus stellatus*			
	From Menai Straits				From Criccieth				From Criccieth			
	a	*b*	*c*†	*d*†	*a*	*b*	*c*	*d*	*a*	*b*	*c*	*d*
0	4·3	3·6	14·1	13·0	3·7	5·0	3·1	4·2	2·6	3·7	3·1	2·9
15	—	—	4·8	4·9	4·1	4·5	—	3·2	2·9	3·0	1·6	4·1
30	G	G	G	G	2·7	2·3	G	G	G	G	0·5	G

B. Oxygen increased by passing the gas through the water at 6 ml./min, temperature 13° C.

Time in min since start	Frequency of individual specimens*												
	Balanus balanoides								*Chthamalus stellatus*				
	From Menai Straits				From Criccieth				From Criccieth				
	w†	*x*†	*y*†	*z*†	*w*	*x*	*y*	*z*	*v*	*w*	*x*	*y*	*z*
0	17·9	11·9	13·9	14·1	3·8	3·5	3·8	3·5	2·1	1·7	2·8	1·3	1·8
15	18·5	C	14·3	9·9	2·3	2·7	2·2	1·8	1·7	1·7	2·6	E	3·2
30	19·3	C	13·7	C	2·3	3·1	2·4	1·1	2·8	4·7	3·3	3·8	4·1
45	18·5	C	C	C	2·3	2·9	2·6	C	2·7	3·7	4·0	E	4·2
	Only one out of 17 of this group open												

Notes. C, terga and scuta tightly closed; G, terga and scuta open, cirri inactive, and in some, protruding E, cirri remaining extended for longer than usual during open phase of beat.

* As beats per 10 sec.

† Specimens showing the fast type of beat.

normal type, and may thus be the first to be inhibited by severe oxygen lack.

The two species of barnacles reacted differently to an increase in the oxygen content of the water. In five specimens of *Chthamalus* the mean frequency of beating increased from 1·94 to 3·75 beats/10 sec, a gain of nearly 90%. The frequency of *Balanus balanoides*, on the other hand, slowed down, the mean of the normal beat decreasing from 3·6 to 2·6 beats/10 sec, while all but one of the specimens showing the fast type of beat gave the closing response.

The more rapid beat of *Chthamalus* in oxygen-enriched water suggests that the species is adapted to an oxygen content higher than that of the laboratory sea water supply, which may not have been fully saturated with air. In Britain this barnacle is most abundant in wave-beaten places, where the water will nearly always be fully saturated. The cessation or slowing down of both types of beat in *Balanus balanoides* suggests that in these specimens oxygen lack was the main driving factor behind cirral activity. From this assumption it could be argued that the fast type of beat was in fact respiratory in function.

The problem of the fast type of beat therefore remains unsolved by these experiments. It is hoped that it will be dealt with again in a later paper in this series.

Discussion

The studies described here and in Part I take no account of possible inherent diurnal rhythms in the behaviour of barnacles. Diurnal rhythms, generally related to the tidal cycle, have been shown to occur in the rate of feeding, respiration, and other processes in many intertidal (and sublittoral) animals, and may persist even after periods of continuous immersion in the laboratory (literature in Rao, 1954). Although during the present work no signs of diurnal variations in behaviour were noticed, the possibility of their existence in barnacles cannot be discounted. However, in the investigations into the effects of tide-level and habitat on cirral activity, any diurnal rhythms would not have affected the comparisons of different samples of barnacles, as the latter were taken practically simultaneously, treated identically in the laboratory, and examined at about the same time of day and period of tidal cycle.

Segal, Rao & James (1953) have shown differences in the rate of activity of limpets and mussels from different tide-levels in California. They suggest that the differences are related to similar differences found in examples of the species from different latitudes, and that the greater activity, at a given temperature, of the forms from low water is comparable to the greater activity of cold-adapted forms from higher latitudes. This is, of course, possible, since animals living at low water in relatively warm latitudes, and exposed to the air for only a short time, will have a more uniform and cooler environment than those at high water that are exposed to the heat of the sun and air for longer periods. But it must be noted that, except for activities such as heart beat, most of the other activities of an intertidal animal (feeding, movement of feeding or respiratory appendages, gaseous exchange) take place mainly under water, when the conditions governing the high- and low-water individuals will be almost identical.

However, at first sight the suggestion of Segal *et al.* might seem to explain the greater cirral activity of the British specimens of *B. balanoides* from low water. As was shown in Part I, this barnacle is at its southern limits in Britain, and its behaviour here must represent its extreme limit of adaptation to higher temperatures, i.e. there is probably ample scope for cold-adaptation by those forms living at low water. At the same time the absence, except in one instance, of any difference in the behaviour of *Chthamalus* from different tide-levels could be due to this species having already, at high water, reached its limit of adaptation to low temperature.

Attractive as this explanation might seem, since it would link up with the restriction of *Chthamalus* to high water and of *Balanus balanoides* to lower down the shore at their respective extreme limits of distribution, it does not fit all the observed facts. The differences in the behaviour of barnacles from different habitats, and the acclimatization that can occur in the laboratory, suggest that, in these animals, the tidal differences are not entirely a matter of temperature. It is well known that the rate of growth of intertidal animals is slower at high water than further down the shore, and that correspondingly their life is longer at high water. It seems possible that the whole life and vital activities of the animal living at high water might be keyed to the slower tempo, and that not only growth, but rate of feeding, respiration, and other processes would therefore be slower than at low water.

Finally, it seems worthwhile pointing out the need for caution in comparing the behaviour or activity of intertidal animals from different tide-levels or latitudes until the effect of other habitat variations have been thoroughly investigated.

SUMMARY

The frequency of beating, and other aspects of cirral activity, were observed in samples of three species of intertidal barnacles taken from various habitats and from different tide-levels.

At Plymouth a more rapid beat was found among individuals of *Chthamalus stellatus* from low water at one locality than among those from high water at the same place. At two other localities, however, there was no significant difference in behaviour between samples from high and low water. A difference was found in samples of *Elminius modestus*, those from low water having a more rapid cirral beat than those from high water.

There was again no difference in the frequency of beating of the cirri of *Chthamalus* collected near Anglesey. Samples of *Balanus balanoides* from the same district, however, with one exception, showed a greater frequency of beat

among those individuals from low water, and had more regular cirral activity, than individuals from high water. Among the specimens from the exceptional locality, which was sheltered from wave action and currents, nearly all the high-water individuals showed the fast type of beat, while the low-water individuals showed only the normal beat.

It was suggested that the fast type of beat was an adaptation to assist feeding or respiration in still water. Experiments with varying oxygen content of the water failed to show whether or not the adaptation was respiratory.

The work suggests that the older a barnacle becomes, the slower is its cirral beat, and the less often it shows cirral activity.

The difference in activity of animals from high- and low-tide levels may be related as much to the difference in rate of growth, and possibly of general metabolism, as to any adaptation to temperature differences between the two levels.

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A SUGGESTED METHOD FOR THE ASSAY OF VITAMIN B_{12} IN SEA WATER

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(Text-figs. 1–3)

One of the difficulties encountered in the use of fresh water organisms for the assay of vitamin B_{12} of marine origin is the elimination of salt from the extracts. This has suggested that a marine organism might be used with advantage. Indeed on several occasions marine or supra-littoral species have been proposed as possible assay subjects, but acceptable techniques await development (Lewin, 1954; Droop, 1954; Sweeney, 1954). *Monochrysis lutheri*, a supra-littoral euryhaline species, has advantages over others in being small, robust and giving heavy yields in synthetic media. The method employed in some *Monochrysis* assays carried out in the early spring of 1954 will be described.

Principle

If a sample of sea water to be assayed, containing Z mμg B_{12}/l., is divided into portions A and B and A is diluted r times with a B_{12}-less synthetic sea-water substitute, the difference in B_{12} content of the two preparations then is

$$Z\frac{r-1}{r}. \tag{1}$$

If, now, each of these preparations is given the same graded doses of B_{12} the regressions of the responses so obtained can be compared and the value of Z ascertained. In Fig. 1

$$Z=\frac{rM}{r-1}. \tag{2}$$

For a parallel line method, such as this, the doses require to lie on that region of the response curve where linear increase of dose causes linear increase of yield measurements. When the optical measurement used is per cent absorption, this region is between doses of 3 and 18 mμg B_{12}/l. for *Monochrysis*.

The choice of three dose levels, say 3, 6, 9 mμg B_{12}/l., with replications, gives a symmetrical six-point layout which can be treated statistically in the manner described by Finney (1952) for parallel line assays. That is, tests can be made for 'regression', 'difference between preparations', 'linearity' and 'divergence from parallelism', and fiducal limits of the estimate can be calculated.

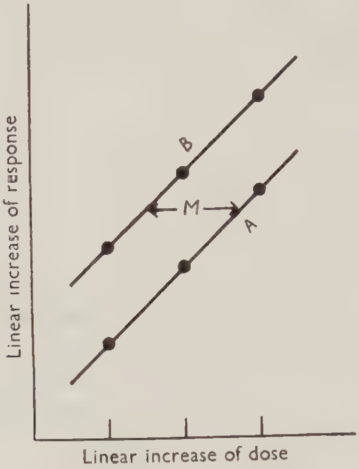

Fig. 1. Diagram of linear parallel line assay. Linear increase of response (vertical) against dose (horizontal) for preparations *A* and *B*. *M* (measured horizontally), difference between regressions in terms of dose.

Preliminary Assay

When the expected value of *Z* is entirely unknown it is necessary to perform a preliminary assay in order to ensure a convenient value for *M*. (*M* can be reduced by diluting the whole sample.) A very rough idea of *Z* can be obtained by adding to a pilot sample the ingredients (less the water) of the nutrient solution given on p. 437, and comparing the growth obtained with typical *Monochrysis* dose response curves (Fig. 3; Droop, 1954). For expected values of *Z* up to 10 mμg/l. no dilution of the original sample is necessary other than the twofold one performed by the addition of the nutrient solution (see below). But for larger expected values of *Z* a proportional further dilution of the original sample should be made.

A value of *r* of less than 3 is too small for convenience and one much greater tends to cause a lack of parallelism between the two regressions. This is occasioned by the fact that autoclaved enriched natural sea water is not as favourable a medium as the enriched synthetic sea-water.

Experimental

For the detailed assay a sample of 200 ml. is large enough. The predetermined initial dilution is made if necessary and the sample is divided in the ratio 3:1. The smaller portion is then diluted threefold. Dilutions are effected with the following freshly prepared artificial sea water:

NaCl	30 g
$MgCl_2 6H_2O$	5 g
KCl	0·75 g
$CaSO_4$	1 g
H_2O (glass distilled) to	1 l.

The two portions are then mixed with their own volumes of the following freshly prepared nutrient solution and the pH adjusted to 7·5:

KNO_3	200 mg
K_2HPO_4	20 mg
Thiamin	1 mg
'T.M.2'[1]	20 ml.
'S.W.2'[1]	10 ml.

Tris-(hydroxymethyl)-aminomethane 1 g.
Glass distilled water to 1 l.

There are now two solutions, one (*A*) containing one-third as much of the original sample as the other (*B*). These solutions are then decanted into test-tubes in 6 ml. aliquots and vitamin B_{12} added as follows:

18 $\mu\mu$g	to each	of four	tubes containing	solution	*A*
18 $\mu\mu$g	,,	,,	,,	,,	*B*
36 $\mu\mu$g	,,	,,	,,	,,	*A*
36 $\mu\mu$g	,,	,,	,,	,,	*B*
54 $\mu\mu$g	,,	,,	,,	,,	*A*
54 $\mu\mu$g	,,	,,	,,	,,	*B*

The tubes are plugged and autoclaved for a few moments at 15 lb. and inoculated with 0·02 ml. of a stock culture of *M. lutheri* and incubated at room temperature (16–22° C.) in continuous 'warm-white' fluorescent light of 100 ft. candles until growth is completed.

Example Assay

All assays of samples of surface water taken in February and March 1954 from Keppel Pier, Millport, on incoming tides, gave results lying between 5 and 10 mμg B_{12}/l. A single example will, therefore, be sufficient to illustrate the method.

Preliminary experiments had suggested that the water was unlikely to yield more than 10 mμg B_{12}/l., so preliminary overall dilutions were not necessary.

The sample, taken from Keppel on 5 April 1954, was assayed immediately.

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[1] For compositions of these metal solutions see Droop (1955).

The results are shown in Table I and Fig. 2. Inspection suggests that there is a difference of $M=2{\cdot}4$ mμg/l. between the two regressions.

TABLE I. ASSAY OF VITAMIN B_{12} IN SEA WATER

The sample is attenuated by a factor of 6 in preparation *A* and one of 2 in preparation *B*.

	Dose 3 mμg/l. B_{12}				Dose 6 mμg/l. B_{12}				Dose 9 mμg/l. B_{12}			
A	30	33	26	36	43	48	42	36	63	54	50	57
B	42	44	34	41	59	54	48	52	70	61	62	67

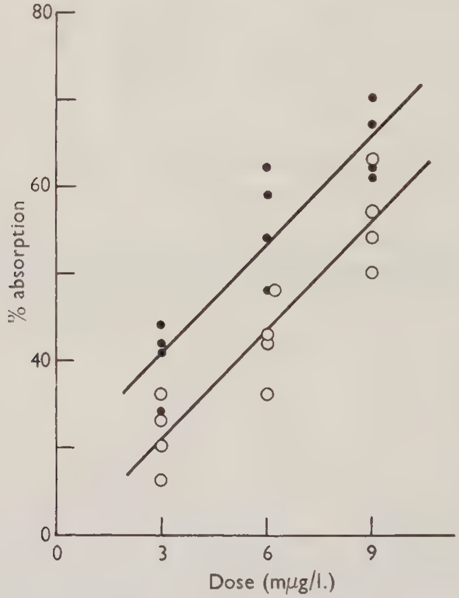

Fig. 2. Assay of Keppel Water, 5 April 1954. Dose of vitamin B_{12} in mμg/l.

$$Z=\frac{rM}{r-1}d, \tag{3}$$

where *d* represents total overall dilutions, including that effected on addition of the nutrients. Substituting,

$$Z=\frac{3\times 2{\cdot}4}{3-1}\times 2$$

$$=7{\cdot}2.$$

The calculated value of *M* is obtained from the equation

$$M=\frac{\bar{y}_B-\bar{y}_A}{b}-(\bar{x}_B-\bar{x}_A),$$

where $b = \frac{\Sigma S_{xy}}{\Sigma S_{xx}}$ (Finney, 1952), which when evaluated from the results set out in Table I, gives

$$M = 2{\cdot}344.$$

In the symmetrical assay both the calculation of M and the arithmetic for the analysis of variance are much simplified by making use of orthogonal contrasts afforded by the layout as described by Finney (1952).

The analysis of variance of the example, set out in Table II, shows that the assay is satisfactory as far as 'difference between preparations', 'regression' and 'linearity' are concerned, but the variance due to 'deviations from parallelism' is too small.

TABLE II. ASSAY OF VITAMIN B_{12} IN SEA WATER. ANALYSIS OF VARIANCE.

Source	D.F.	Sums of squares	Mean square
Preparation	1	560·7	560·7
Regression	1	2450·3	2450·3
Parallelism	1	0·0	0·0
Quadratic	1	0·75	0·75
Difference of quadratics	1	5·3	5·3
Between doses	5	3017·1	603·4
Error	18	290·9	16·2
Total	23	3308	

The 95% fiducal limits to M are obtained by evaluating

$$\left[M-\bar{x}_A+\bar{x}_B \pm \frac{ts}{b}\left\{(1-g)\left(\frac{1}{N_A}+\frac{1}{N_B}\right)+\frac{(M-\bar{x}_A+\bar{x}_B)^2}{\Sigma S_{xx}}\right\}^{\frac{1}{2}}\right] \div (1-g),$$

where $g = \frac{t^2 s^2}{b^2 \Sigma S_{xx}}$ and the value of t for a 0·05 probability and 18 degrees of freedom is taken as 2·101 (Finney, 1952).

The limits to M are thus 3·357 and 1·471. Substituting in (3) we get for the B_{12} estimate of the sample: 7·03 mμg/l. with limits 10·07 and 4·41.

DISCUSSION

Monochrysis responds to pseudo-B_{12}, and factor *A* but not to factor *B* (Fig. 3) so that its specificity can be compared to that of *Lactobacillus leichmannii*.

The assay method described here has some advantages and some obvious disadvantages. It is reasonably sensitive and admits of statistical treatment, and its design is such as to eliminate all carry over effects, but its greatest advantage is that it is direct in the sense that it involves no extraction procedures which require much time and large samples and, moreover, are possibly subject to errors due to loss of the vitamin. Desalting methods (Provasoli & Pintner, 1953) are at present subject to similar errors. However, the advantages of the *Monochrysis* method are offset by the length of time taken for the assay to complete. *Monochrysis* is an obligate phototroph with a generation time of about 24 h, with the consequence that cultures take about 3 weeks to mature. It is, therefore, certain that the *Monochrysis* method would never be used with advantage for routine purposes, though the possibility of its being useful in some instances is envisaged.

Monochrysis is one of many B_{12}-requiring marine phytoflagellates which can be handled in a similar manner. A number of such species with differing specificity patterns employed concurrently with the different forms of the vitamin could provisionally yield useful information of the B_{12} content of sea water. *Prymnesium parvum* or *Syracosphaera elongata*, for instance, are as specific as *Ochromonas* in their response.

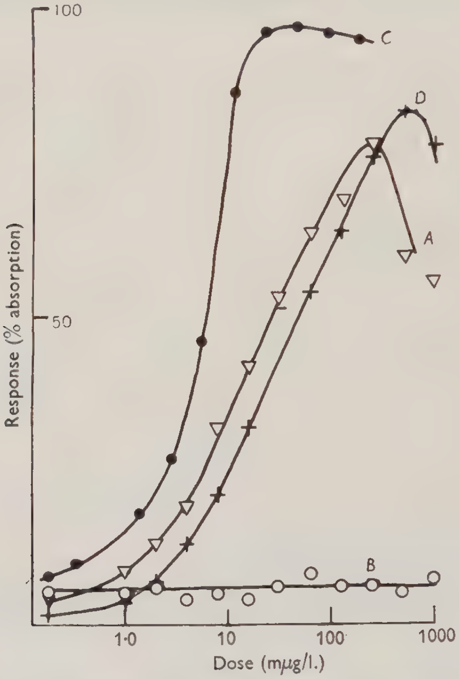

Fig. 3. Response of *M. lutheri* to vitamin B_{12} (curve *c*), factor *A*, pseudo-vitamin B_{12} (curve *D*), and factor *B* in presence of adenine.

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THE CYPHONAUTES LARVAE OF THE PLYMOUTH AREA AND THE METAMORPHOSIS OF *MEMBRANIPORA MEMBRANACEA* (L.)

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From the Plymouth Laboratory

(Text-figs. 1–4)

Several cyphonautes larvae (Polyzoa Ectoprocta) have been distinguished and described (Lohmann, 1911; Marcus, 1940; Thorson, 1946), but few have been seen to metamorphose, thus linking the late larva with the adult. Three, belonging to the suborder Cheilostomata, which have been observed to do so, are *Cyphonautes compressus* into *Electra pilosa* L. (Schneider, 1869; Barrois, 1877; Kupelwieser, 1905–6), *Cyphonautes occidentalis* into *Membranipora villosa*, (Robertson, 1908; O'Donoghue, 1927) and an unnamed cyphonautes into *Nichtina* (= *Membranipora*) *tuberculata* (Hastings, 1929, pp. 706–7).[1]

Prouho (1892) found *Alcyonidium albidum* and *Hypophorella expansa* (Ctenostomata) to have cyphonautes larvae, but was unable to rear them to the stage when pyriform and adhesive organs were present, so that the appearance of the late larvae of these two species is unknown. He also reared the cyphonautes of *Electra pilosa* to a young stage, and took later larvae from the plankton (pl. 30, fig. 92). *Alcyonidium albidum* has not been recorded in the Plymouth area; *Hypophorella expansa* has been recorded (Marine Biological Association, 1931, p. 289), but the cyphonautes has not yet been recognized.

During work on the ciliary feeding mechanism of the cyphonautes larva (Atkins, 1955), two species were taken from the plankton in the Plymouth area, one large and one small, and both seen to metamorphose and give rise to colonies.

Inside Plymouth breakwater the common form in the tow-nets throughout 1953 was a small, rather opaque, cyphonautes, which developed into *Electra pilosa* L. and is evidently that known as *Cyphonautes compressus*.[2] It is about 440 μ broad at the base and 360 μ high when ready to metamorphose;

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[1] For discussions on the validity of *Membranipora* as the name for the genus see Borg (1931) and Osburn (1950).

[2] In the autumn of 1953 a small number of the larvae of *Electra pilosa* were seen to be parasitized by young stages of a species of *Endodictyon*, probably close to *E. infestans* Gran, which is known to be endozoic in species of *Alcyonidium* (Newton, 1931, p. 128). It would seem probable that the colonies of *Electra pilosa* are parasitized and that the larvae carry the infection. The identification was kindly made by Dr M. Parke.

metamorphosis may, however, occur when the shell is only about 390 μ broad. Young forms with inhalant and exhalant chambers fully formed are no more than 160 μ broad and 130 μ high. The valves of the late larva are of a brownish tint, but are without ornamentation along their basal edges. Fig. 1 shows change in shape of the larva of *Electra pilosa* with growth. These stages have been taken from the plankton and not reared, but as there is considerable difference in size and transparency between the large cyphonautes and the larva of *E. pilosa* the young stages of the two are unlikely to be confounded and the late stages never (cf. Figs. 1 and 3 which are reproduced at the same magnification).

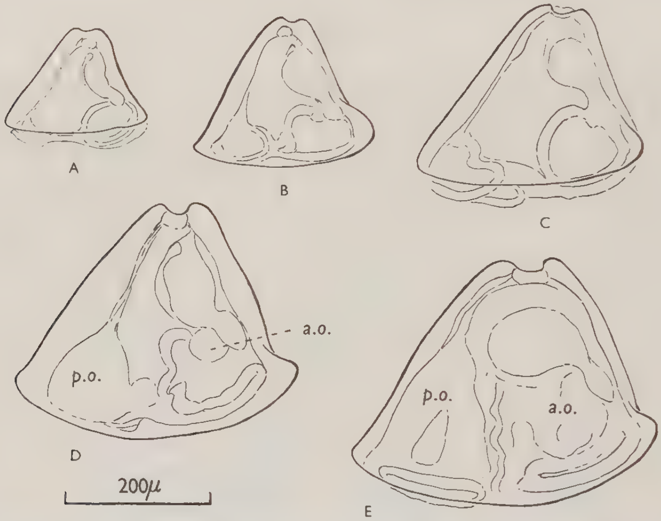

Fig. 1. *Electra pilosa* L. Cyphonautes larva (*Cyphonautes compressus*), to show change in the shape of the shell with growth, and the development of the pyriform (*p.o.*) and adhesive (*a.o.*) organs.

The first cyphonautes to be described, the *Cyphonautes compressus* of Ehrenberg (1838), was apparently lacking in ornamentation even in the late stage, for none is shown in his figure (pl. 44, fig. 2) of a late larva with well-developed pyriform and adhesive organs. He gave the size of this larva as '$\frac{1}{9}$ linie', which as the German line equals 2·18 mm, is about 242 μ. Schneider (1869) was quite possibly right in identifying his larva, which he found to develop into *Electra pilosa*, with *Cyphonautes compressus* Ehrenberg.

The settlement of *C. compressus* has been watched several times at Plymouth, for late larvae when taken from tow-nettings and placed in glass dishes tend to settle rapidly. A larva when about to settle glides over the substratum with the pyriform organ foremost, the vibratile plume clawing at the surface. The corona is extended, the cilia at first beating metachronally, although slowly:

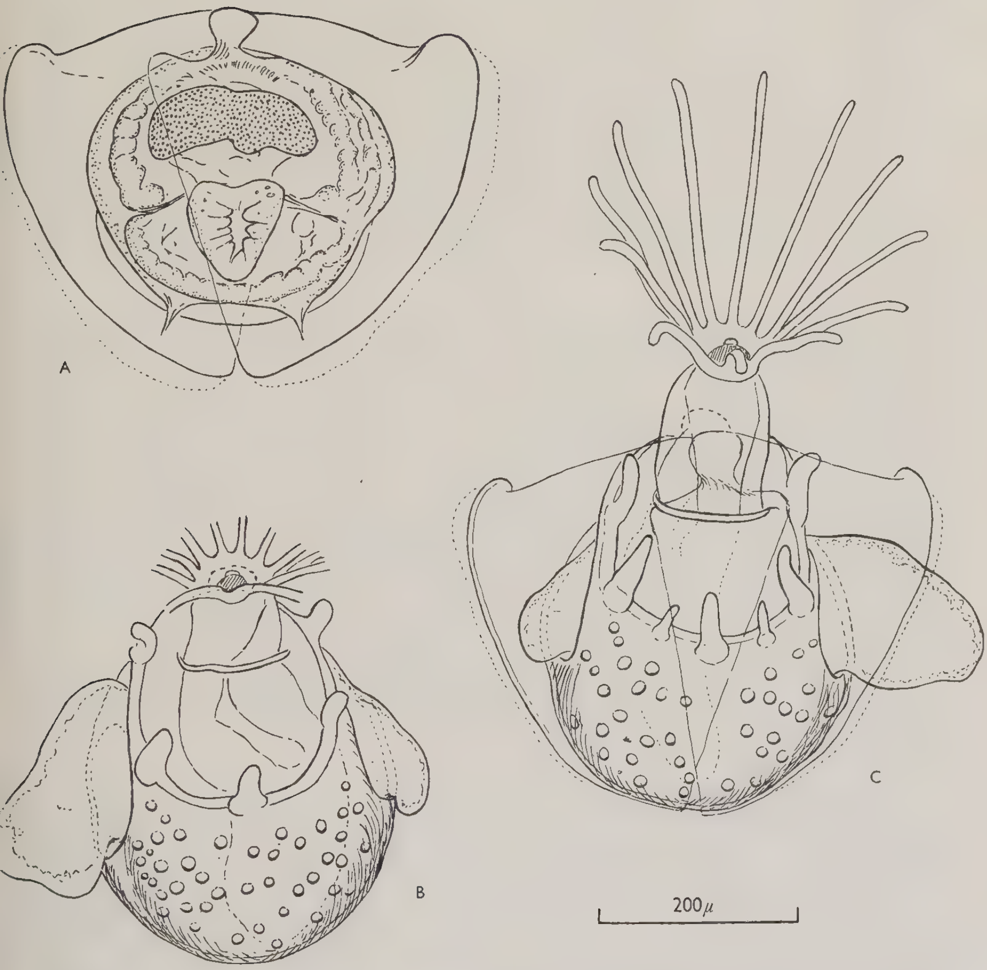

Fig. 2. *Electra pilosa* L. A, metamorphosing larva; B, ancestrula with five spines and the polypid with eleven tentacles; C, ancestrula with seven spines and the polypid with twelve tentacles. The shell is shown in position in A and C; it was removed from B for the figure to be drawn. Both ancestrulae have given rise to three buds, two being lateral and one distal (the latter is indicated by a broken line).

later the cilia become quiescent, extended almost horizontally. When gliding, if gently squirted at with a pipette, it heels over, but is not dislodged: when touched with a needle it can be seen to be attached only by the pyriform organ, which secretes some adhesive substance, for when forcibly detached from the substratum a thread of secretion is visible. Immediately before settlement the cyphonautes moves around in circles, then suddenly the valves separate and flatten and the larva is strongly attached and can only be detached by scraping. While the anterior edges of the valves remain united by the mantle, the posterior edges separate, the gap between them widening, as the valves spread out, and when this is completed the two anterior margins of the valves come to overlap. Many larvae have settled in a reversed position beneath the water film.

The development of the young colony of *Electra pilosa* from the cyphonautes larva has been described and figured by Schneider (1869, pl. 16, figs. 2–7) and Barrois (1877), and it is known that on metamorphosis the larva gives rise to a single zooecium or ancestrula. The development of the colony from the ancestrula has been described by Waters (1924) and Marcus (1926, p. 31). A figure of the ancestrula of *E. pilosa* (Fig. 2) is given here for comparison with that of *Membranipora membranacea* (L.) (Fig. 4). Most of the ancestrulae of the Plymouth *Electra pilosa* had five spines and their polypides had eleven tentacles, but those of one settlement had two small extra spines and the polypides had twelve tentacles (Fig. 2). The gymnocyst was always perforated, although a few specimens had only a small number of perforations.

In larvae which metamorphosed on the surface film and were then pushed to the bottom of the dish, but did not come to rest in the normal position, the three buds from the ancestrula grew long, narrow and sharply curved in seeking to reach the substratum.

Outside the breakwater the common form in 1953 was a large, transparent cyphonautes (*Cyphonautes compressus* was rarely found outside in that year although it was found in October 1954), attaining a width of 840 μ and a height of 640 μ when ready to metamorphose; however, metamorphosis may occur when the valves are only 750 μ broad. When young the shell is colourless and without ornamentation; the valves are almost as high as wide at the base. At about 710 μ basal width the ventral edges of the valves become brownish, but there is still little or no ornamentation. With development of the pyriform organ (Fig. 3, *p.o.*), which is formed before the adhesive organ (*a.o.*), the anterior edge becomes increasingly longer than the posterior, and the greatest width considerably more than the height of the shell. With age also the basal edges of the valves take on a brown tint, and become progressively ornamented with refringent spots which in places appear to coalesce, forming ridges. The shell ornamentation is heavier in some metamorphosed larvae than in others. The posterior margin of the shell usually is almost invisible. Fig. 3 shows change in shell shape with age. These stages have been taken from the plank-

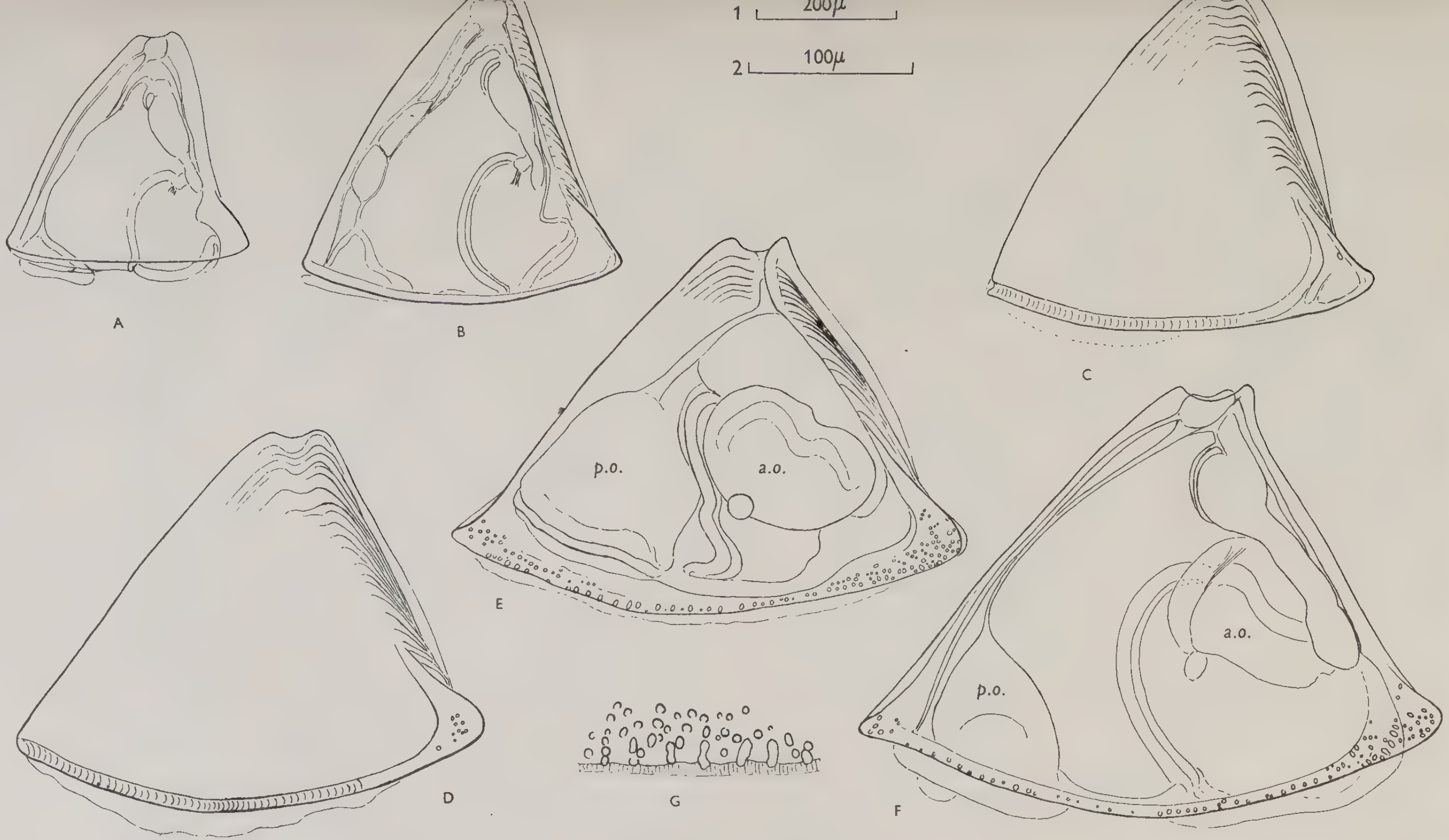

Fig. 3. *Membranipora membranacea* (L.). Cyphonautes larvae, to show change in shell shape with growth and the development of the pyriform (*p.o.*) and adhesive (*a.o.*) organs, and also the appearance of ornamentation along the basal edge of the shell with age. Both the larvae shown in E and F are about ready to metamorphose, although one is smaller than the other. G, small area of the basal edge of the shell enlarged to show the ornamentation and the almost transparent, faintly striated, extension of the shell edge. The valve only is shown in C and D, so that the growth lines are clearly visible. A–F to scale 1; G to scale 2.

ton and not reared.[1] This larva is probably the *C. compressus* of Claparède (1863, pl. xviii, figs. 15–18), if his figures represent stages in the development of one larva, which they appear to do, but it is certainly not the *C. compressus* Ehrenberg (1838) which Schneider (1869) found to develop into *Electra pilosa*. Lohmann (1911) separated Claparède's four figures into three species, *Cyphonautes compressus* Eh., *C. schneideri* Lohm. and *C. borealis* Lohm. Claparède did not give the magnification of his figures, but if all were drawn to the same magnification, then his figs. 15 and 16 are unlikely to be of *C. compressus* Eh. on account of their size alone.

According to Harmer (1926, p. 200) statements in the literature that a cyphonautes has been found in *Membranipora membranacea* (L.) (=*Nichtina telacea*) refer principally, if not exclusively, to *M. mülleri* Bidenkap (=*Electra pilosa* forma *membranacea*, and now known as *Electra crustulenta* (Pallas), see Borg, 1931, p. 11), and he had been unable to find evidence of its occurrence in the British species commonly known by that name. Thorson (1946, pp. 158–9, fig. 87B) mentioned that a cyphonautes, lacking ornamentation in the oldest stage, and which he referred to *Cyphonautes balticus*, metamorphosed in his dishes and was 'presumably the larva of *Membranipora membranacea* (L.)'. The present work shows this assumption to have been wrong, for the large Plymouth cyphonautes with ornamented shell was observed to metamorphose into *M. membranacea* (L.) and this appears to be the first definite observation that this species has a cyphonautes larva. The larva seems to be closest to Schneider's third species (1869, pl. 16, fig. 11), which Lohmann (1911) named *Cyphonautes schneideri*. Schneider's second species (pl. 16, fig. 10), also with an ornamented basal edge, which Lohmann named *C. borealis*, appears to have the posterior edge of the shell almost at right angles to the base, thus differing from the larva which develops into *Membranipora membranacea*. It is possible, however, that this difference in the angle is an individual variation, and the two species are not distinct.

The larva of *M. membranacea* gives rise to twin zooecia while still covered by the flattened valves (Fig. 4A). Nitsche (1871, pl. 38, fig. 3), and Waters (1925) have shown that 'a double ancestrula' occurs in this species, and the latter author figured a young colony of about twenty-two zooids (pl. 21, fig. 1), but neither of them found or described the larva. The twin ancestrulae of young colonies reared at Plymouth do not quite agree with the description given by Waters in that there may be fewer than '4 stout protuberances', but they also differ slightly among themselves (see Fig. 4). The paired proximal tubercles in the colony of seven fully-formed zooids have been pushed on to the undersurface of the colony and one only is visible (Fig. 4F).

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[1] Dr A. B. Hastings has informed me by letter that in July 1930 she kept a few larvae of this species alive for a week and noted the development of the tuberculations (which were absent at first) and their increase in numbers. She also noted the increase in length of the anterior edge and associated sharpening of the anterior angle of the shell.

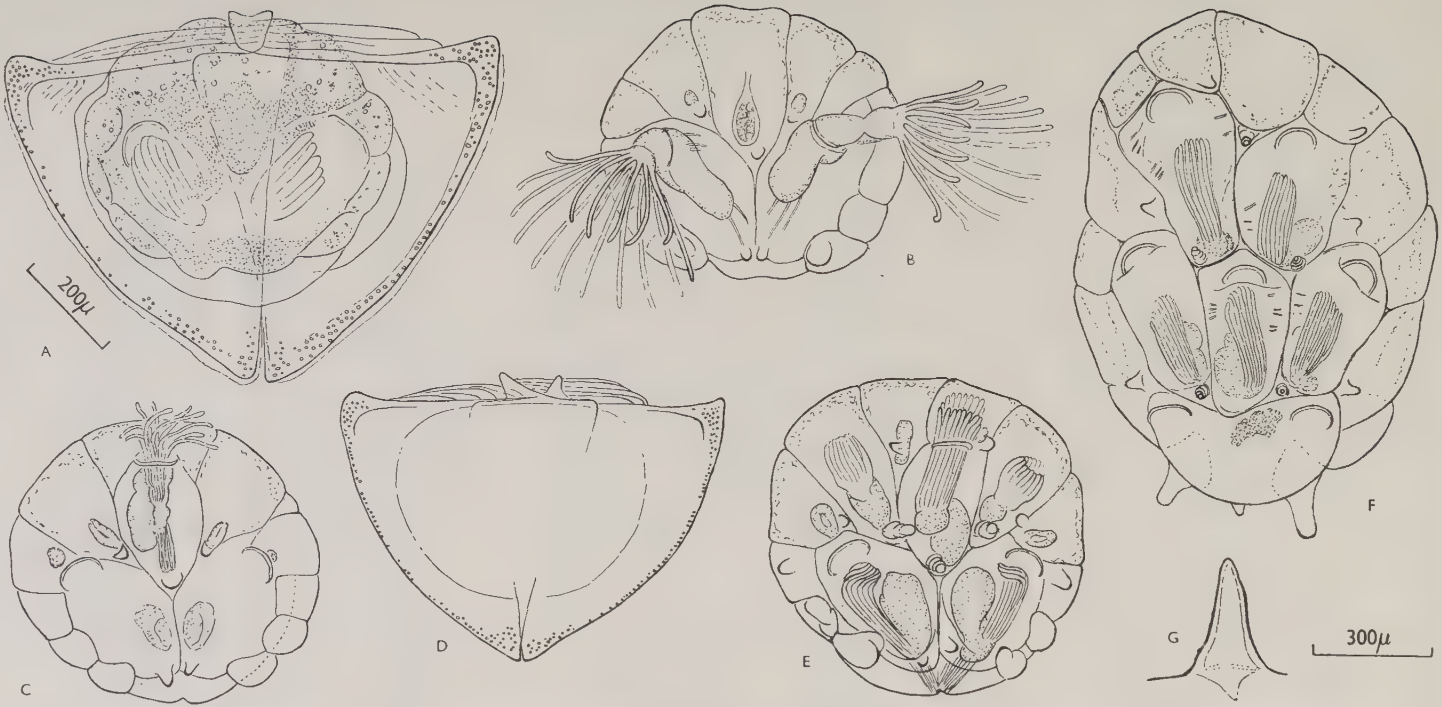

Fig. 4. *Membranipora membranacea* (L.). A, metamorphosing larva; B, C, E, F, development of the colony. G, spine in side view, enlarged. Shells were removed from colonies B and E before drawing; that from colony E is shown at D. The slight asymmetry of the colony shown in F is due to injury in removing it from the bowl in which it grew. B–F to scale in right-hand lower corner.

The development of the young colonies of *M. membranacea* closely resembles that of *M. villosa* Hincks as figured by Robertson (1908) and O'Donoghue (1927) and of *M. tuberculata* (Bosc) as figured by Hastings (1929). These two latter species are known also to have cyphonautes larvae with ornamentation along the basal edges of the valves, described variously as 'teeth' (Robertson, 1908), 'small spine-like denticulations' (O'Donoghue, 1927) and a 'tuberculated edge' (Hastings, 1929), and give rise to twin ancestrulae. As pointed out by Hastings (1929, p. 707) 'the occurrence of twin ancestrulae in three of the six species referred to *Nichtina* (= *Membranipora*) by Harmer (1926, p. 208), the ancestrulae of the other three being unknown, suggests that this may prove to be a generic character'; it would also seem that ornamentation of the basal edges of the valves of the cyphonautes may be a generic character.

The ribbing of the shell parallel with the posterior edge referred to by some authors (Hastings, 1929, p. 707) is due to growth lines and these are shown in Fig. 3C–E.

The work recorded in this note was incidental to a study of ciliary feeding mechanisms, undertaken while holding a Leverhulme Research Fellowship. Dr A. B. Hastings has most kindly read the note and drawn my attention to the papers by Borg (1931) and Osburn (1950).

SUMMARY

The cyphonautes larvae of *Electra pilosa* L. and of *Membranipora membranacea* (L.) have been identified at Plymouth. Change in shell shape of both larvae during growth is described and figured from specimens taken from the plankton. Their metamorphosis has been observed, that of the larva of *M. membranacea* (L.) apparently for the first time, thus definitely proving the presence of a cyphonautes larva in that species.

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THE CILIARY FEEDING MECHANISM OF THE CYPHONAUTES LARVA [POLYZOA ECTOPROCTA]

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(Text-figs. 1–7)

The cyphonautes larva found in certain ectoproct Polyzoa is considered to be most probably a primitive larval form, which has been lost in viviparous species, the larvae of which are degenerate in varying degree and settle within a short time. The cyphonautes is truly planktonic and has a free swimming life of some length—of 2 months according to Marcus (1940, p. 334)—and unlike the other known larvae of the group, has a functional alimentary canal. It obtains its food by maintaining a continuous current of water through the mantle cavity or vestibule, its ciliary mechanism being organized on a plan somewhat resembling that of bivalve molluscs, with an inhalant and an exhalant chamber with interposed ciliary ridges bearing current-producing and food-conveying cilia. As in lamellibranchs the organ creating the water and food currents is separated by a considerable interval from the mouth. In lamellibranchs this interval is bridged by the palps and the oral grooves; in the cyphonautes by the ciliated tract of the funnel. The cyphonautes being a larval form of probably archaic character its method of feeding is of particular interest.

For the study of the ciliary feeding mechanism the larva of *Membranipora membranacea* (L.) (= *Nichtina telacea* (Lamarck)) was mostly used, rather than that of *Electra pilosa* L., because of its greater size and transparency. The former larva attains a basal width of 840 μ, while the latter reaches only 440 μ (see Atkins, 1955). For the indication of currents *Chlorella stigmatophora*, *Peridinium trochoideum* and *Phaeodactylum tricornutum* Bohlin were used. They were most kindly supplied by Dr M. Parke.

Larvae for sectioning were narcotized with magnesium chloride, a few drops of 7% in tap water being added at intervals to a watch-glass of sea water. The narcotized larvae were first dropped into 10% formalin for about a minute, and then transferred to a modification of Bouin's fixative (saturated picric acid in 90% alcohol, 2 parts; saturated corrosive sublimate (water), 3 parts; 40% formalin, 1 part; glacial acetic acid, 2 parts). In this way larvae were fixed extended. If dropped directly into the second fixative they were found to contract strongly even after being well narcotized. Sections were cut 4, 6 and 8 μ in thickness.

It was not until my paper had been written that I had an opportunity of seeing the beautifully detailed and illustrated paper by Kupelwieser (1905–6) on the histology and metamorphosis of the larva of *Electra pilosa*.

The Structure of the Cyphonautes Larva with special Reference to the Mantle Cavity or Vestibule

The general structure of the cyphonautes larva is well known (Fig. 1). The body is enclosed in a triangular bivalve shell, chitinoid and transparent, and is strongly compressed laterally. The valves are approximated by an adductor muscle, composed of smooth fibres, which before the adhesive organ develops has the form of an arc; a muscular loop slung round the adductor attaches it to the body wall over the gut.

The apex of each valve is truncated and grooved for the reception of the apical organ, which bears a tuft of long cilia, sensory in function.

The pallial lobes enclose a large mantle cavity or vestibule. The external epithelium between the anterior and posterior edges of the valves in life is minutely papillate, with protoplasmic protrusions. It bears ciliary tufts and long cilia, which are probably sensory in function, for they have a slow and irregular beat.

Basally the valves gape and the pallial lobes are here thickened and heavily ciliated, forming a complete ring or corona in very young larvae according to Prouho (1892, p. 616). In older larvae the cells of the middle region are reduced in height and bear short, inconspicuous cilia, as first noted by Claparède (1863, p. 108). Thus the corona—the swimming organ of the larva—is divided into separate anterior and posterior regions.

The anterior portion of the corona borders the entrance to the pyriform organ, or, in young larvae, the invagination which is the precursor of that organ. At each end posteriorly it is sharply recurved, running inwards and anteriorly toward the glandular region of the pyriform organ.

The posterior portion of the corona borders the exhalant aperture and the strong beat of its cilia, from the apex toward the base of the shell, contributes to the exhalant current.

Reduction of the cilia in the middle region, or inhalant aperture, is no doubt to prevent their action from interfering with the inhalant current. If present in full development, the current produced by them would be in direct opposition to the inhalant current. In young larvae of *Electra pilosa*, with basal width of shell about 160 μ, the inhalant aperture is large compared with the anterior region of the corona.

Two ciliated lobes projecting toward each other across the mantle cavity, separate the middle or inhalant aperture from the exhalant aperture. They are directed slightly downward, beyond the ciliated corona, and posteriorly. Each lobe is continued as a ciliated ridge, ascending in an arc across the

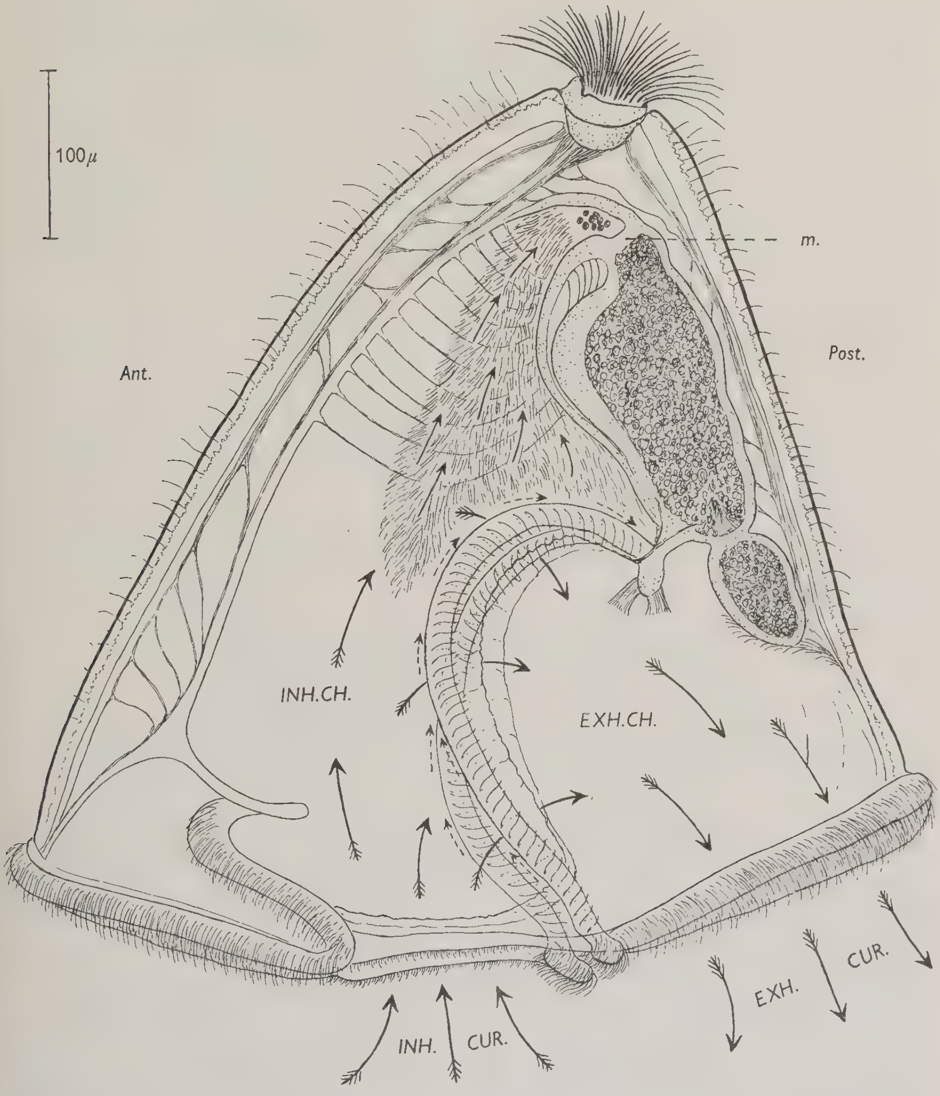

Fig. 1. *Membranipora membranacea* (L.): young cyphonautes larva, to show the direction of the main water current (barbed arrows), of the food currents of the frontal surfaces of the ciliated ridges (small broken arrows) and of the ciliated funnel (small unbroken arrows). The metachronal waves of the lateral cilia of the ridges are indicated. Certain muscles are shown. *INH.CH.* and *EXH.CH.*, inhalant and exhalant chambers. *INH.CUR.* and *EXH.CUR.*, inhalant and exhalant currents; *m*, position of oesophageal mouth.

mantle wall: at their inner ends the two ridges are continuous. The paired ciliated ridges thus divide the mantle cavity into two, an anterior inhalant chamber and a posterior exhalant chamber, or, expressed in another way, form the border of an aperture between the two.

The inhalant chamber extends from the base to the apex of the shell, and at its apical end lies the entrance to the oesophagus, which may be termed the oesophageal mouth. The anus opens into the exhalant chamber. As the larva approaches metamorphosis the development of the pyriform organ greatly reduces the size of the inhalant chamber, and the development of the adhesive organ the exhalant chamber. Also various patches of glandular (?) tissue develop, probably in connexion with metamorphosis.

The shell is composed of two layers. The outer layer is exceedingly thin and fades out basally; it stains black with iron haematoxylin and red with Azan. The inner layer stains faintly with iron haematoxylin and dark blue with Azan. The basal edge of each valve is strengthened with a continuous band of material staining black with iron haematoxylin and red with Azan. In life a transparent extension of the shell edge basally is apparently for the protection of the extended corona; this may be the portion which in sections stains light blue after 40–50 seconds in alcian blue and also with Azan (see Figs. 3, 4).

THE CILIATION OF THE MANTLE CAVITY

The Inhalant Chamber

The inhalant aperture (Figs. 2, 3 A) is bordered by a delicate velum, which is incomplete posteriorly between the bases of the ciliated ridges. This gap can be closed by the apposition of the free lobed ends of the ridges. The velum is composed, except anteriorly, of a single row of cells, each bearing about fifteen long cilia arranged in a fan-shaped group; the outer cilia of one group cross those of adjacent groups (Fig. 6 E). These cilia are frequently inactive, and then extend inwards, parallel with the mantle lobes. At other times they have been seen stationary bent from their bases outwards, with the tips pointing toward the exterior, and at others curved or looped about the middle of their length. From such positions they straighten rapidly. They probably act as a sieve, as suggested by Kupelwieser (1905, p. 23). In the velum runs a fine muscle strand, which on contraction reduces the size of the inhalant aperture, the large ciliated cells becoming corrugated. The velar muscles are continuous posteriorly with the longitudinal muscles of the suspensory membranes of the ciliated ridges, while it would appear that anteriorly fibres are given off which are inserted on the pyriform organ. The appearance of the velum where it adjoins the pyriform organ is shown in Fig. 3 B, part of a section passing through the extreme posterior region of the anterior portion of the corona, where the ciliated bands turn inward to run across the ventral face of the larva. Kupelwieser (1905–6, p. 23, text-fig. 7) considered it probable that the velum of *Cyphonautes compressus* inclined outward when relaxed. In the larva of *Membranipora membranacea* it is reflected inward when relaxed, as may be seen in life, and in this position would be likely to interfere less with the ingoing current than in the position suggested by Kupelwieser.

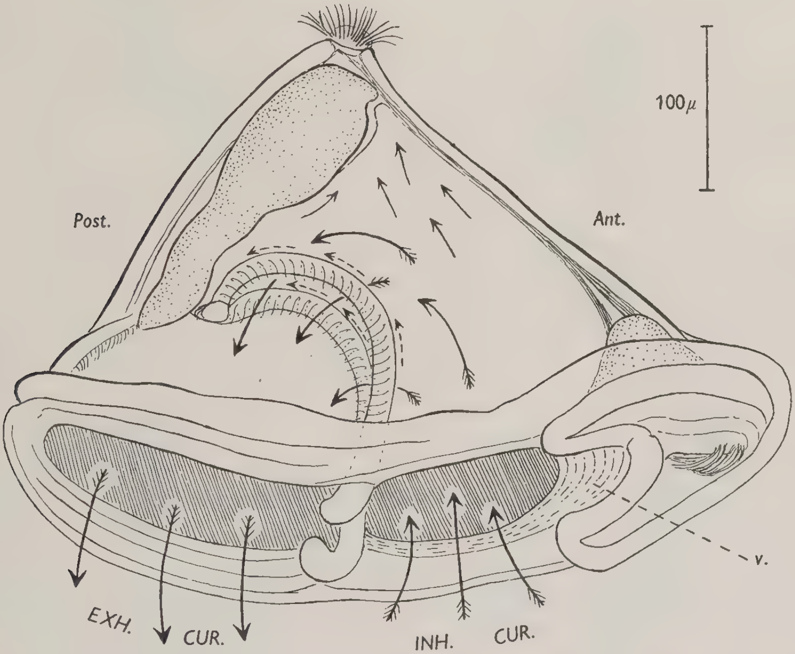

Fig. 2. *Electra pilosa* L.: cyphonautes larva (with pyriform organ well developed, but before the development of the adhesive organ), to show the inhalant and exhalant apertures. *INH.CUR.* and *EXH.CUR.*, inhalant and exhalant currents; *v.*, velum. Arrows as in Fig. 1. Cilia mostly not shown.

At the base of the velum is a ridge of a single row of cells bearing long, coarse cilia (Fig. 3, *c*). These cilia appear active only when stimulated. When at rest they lie with their tips directed anteriorly; their effective stroke is toward the base of the ciliated ridges and they probably function in directing food organisms toward these.

Externally the inhalant aperture is bordered by the much reduced corona (Fig. 3A, *r.c.*), the short cilia of which partake of the activity of the fully developed anterior and posterior portions of the corona and have the same type of metachronal wave, the wave passing uninterruptedly over this region.

The upper part of the inhalant chamber has the form of a highly muscular and ciliated funnel leading to the oesophageal mouth (Fig. 1). Circular muscles are present in its walls and occasionally when the lowest ring only is contracted,

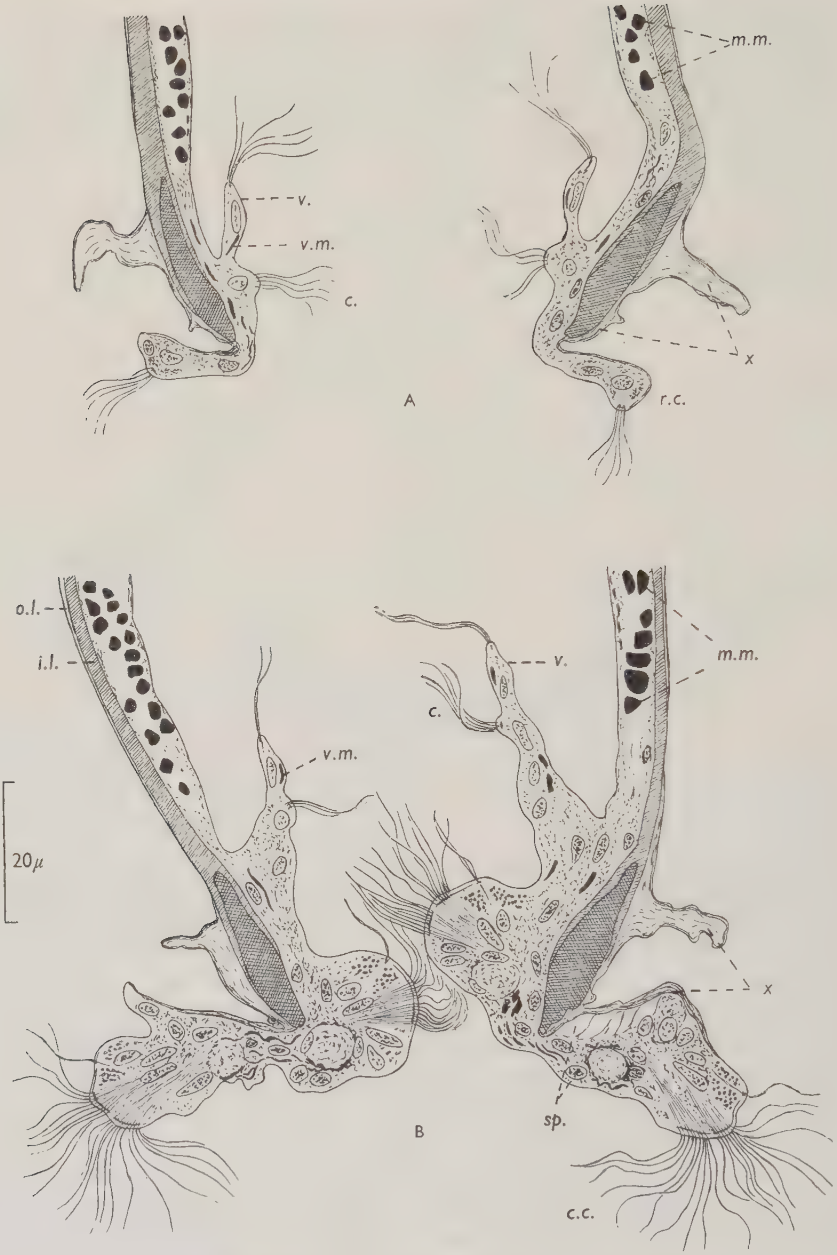

Fig. 3. *Membranipora membranacea* (L.): cyphonautes larva. A: section passing through the inhalant aperture to show the velum (*v.*) and the reduced coronal cells (*r.c.*). The velum is in the relaxed position. B: section passing through the anterior region of the velum (*v.*) near the extreme posterior part of the pyriform organ. The recurving of the corona makes it appear double on each side. *c.*, cilia of the ridge at the base of the velum; *c.c.*, cilia of the corona; *i.l.*, inner layer of shell; *m.m.*, mantle muscles; *o.l.*, outer layer of shell; *r.c.*, reduced coronal cells; *sp.*, spaces due to shrinkage; *v.*, velum; *v.m.*, velar muscle; *x*, region of shell staining light blue with alcian blue and with Azan. The inner layer of the shell is shown hatched and the strengthening band cross-hatched. Heidenhain's iron haematoxylin and alcian blue.

acting as a sphincter, the funnel momentarily has the appearance of an oesophageal or pharyngeal funnel, as remarked by Claparède (1863, p. 107, and pl. 18, fig. 15). In life when fully expanded the 'oesophagus' or 'pharynx' passes indistinguishably into the main part of the inhalant chamber. Contractions of the circular muscles of the funnel appear to occur only when the animal is irritated by the entry into the inhalant chamber of large food organisms, for instance peridinians.

In the cyphonautes of *Electra pilosa* long coarse cilia entirely clothe the funnel and extend well down toward the inhalant aperture. In the cyphonautes of *Membranipora membranacea* the posterior half only of the funnel is ciliated and the ciliation extends as a gradually narrowing tract to a point about half-way down the inhalant chamber (Fig. 1).

The cilia of the funnel may be divided into three regions according to their activity: (1) an outer area over which the cilia are generally inactive unless the larva is feeding, (2) a middle area over which cilia are active at short intervals, and (3) a small inner area over which cilia appear generally active. Immediately in front of the sphincter guarding the entrance to the oesophagus is an unciliated area where food organisms collect before being swallowed or rejected.

The Exhalant Chamber

In the larva of *Electra pilosa* some scattered ciliation is present at the apex of the exhalant chamber and along the base of the suspensory membrane of the ciliated ridges. These cilia are not continuously active. The mantle cilia of the exhalant chamber are not as long as those of the inhalant chamber. In this larva a broad tract of cilia leads from the anus to the mantle edge: the cilia are generally active.

In the larva of *Membranipora membranacea* the exhalant chamber appears to be unciliated, except for cilia clothing the outer surface of the rectum, near the anus. These cilia are fully active only when a faecal pellet is being expelled. Their position of rest is at the beginning of the effective stroke.

The exhalant aperture is bordered by the posterior portion of the corona (Fig. 4), the cilia of which beat from the direction of the apex of the shell toward its base.

Glandular cells of the corona described and figured by Kupelwieser (1905–6, p. 5; pl. IV, figs. 17, 18) for the larva of *Electra pilosa* fixed in Flemming's and Hermann's solutions, were absent in my sections of the larva of *Membranipora membranacea*. It may be that such glandular cells are only present in late larvae ready to metamorphose, or it may be a specific difference, or perhaps the fixative used (see p. 451) may have dissolved the contents.

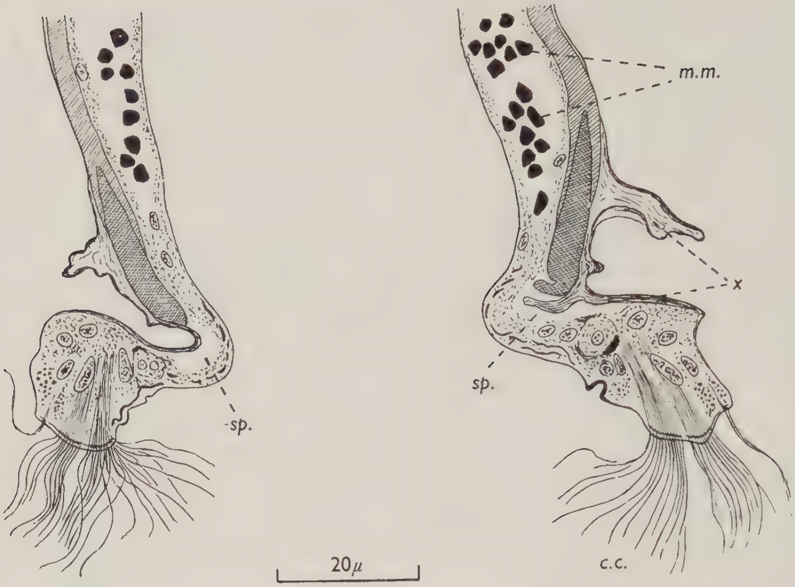

Fig. 4. *Membranipora membranacea* (L.): cyphonautes larva. Section passing through the exhalant aperture. *c.c.*, cilia of the corona; *m.m.*, mantle muscles; *sp.*, space due to shrinkage; *x.*, region of shell staining light blue with alcian blue and with Azan. The inner layer of the shell is shown hatched and the strengthening band cross-hatched. Heidenhain's iron haematoxylin and alcian blue.

The Structure of the Ciliated Ridges separating the Inhalant and Exhalant Chambers

The ciliated region of the ridges is suspended from the pallial lobes by a narrow, delicate, mobile membrane, which becomes contracted on fixation. Along the base of the suspensory membrane runs a muscle strand, which distally is continuous with the velar muscle. Proximally it is inserted on the body wall near the stomach (Fig. 1). At some little distance from its insertion a short branch is given off, which loops round the shell adductor muscle (Fig. 5B). From the longitudinal muscle of the suspensory membrane fine fibres run transversely into the ridges (Fig. 1). In each ciliated ridge is a fine longitudinal muscle fibre (Fig. 6A, *l.m.r.*). The muscular systems of the ciliated ridges, velum, pyriform organ and the adductor muscle are closely connected. The relation of the inner ends of the ciliated ridges to one another and to the shell adductor, before the development of the adhesive organ, is shown in Fig. 5B.

Seen in the living larva, the two curved ciliated ridges have much the appearance of narrow gill filaments, but lateral, current-producing cilia are present on the inner surface only.

On the ridges the ciliated cells are in three tracts, a frontal, a latero-frontal and a lateral (Fig. 6A). This ciliation is continued on to the free, lobed extensions of the ridges and these have a tract of latero-frontal and of lateral cilia on their outer as well as on their inner surfaces. No mucous glands could be distinguished in connexion with the ciliated tracts on the ridges: a group of large clear cells is present in the free lobed ends of the ridges, but these did not become blue when the larvae were placed in water tinted with methylene blue. In sections, spherules staining heavily with iron haematoxylin are arranged peripherally in these cells.

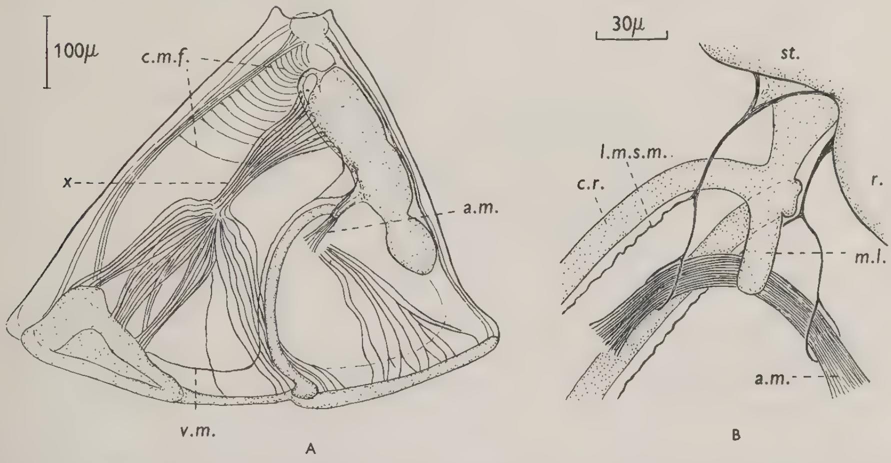

Fig. 5. *Membranipora membranacea* (L.): cyphonautes larva. A: the chief muscles of one mantle lobe as seen immediately after fixation in the fixative given on p. 451. B: sketch to show the relation of the ciliated ridges and their muscles to the shell adductor muscle, as seen in life. *a.m.*, adductor muscle of the shell; *c.m.f.*, circular muscles of the ciliated funnel; *c.r.*, ciliated ridge; *l.m.s.m.*, longitudinal muscle of the suspensory membrane; *m.l.*, muscular loop connecting the adductor muscle to the body wall over the stomach; *r.*, rectum; *st.*, stomach; *v.m.*, velar muscle, which continues as the longitudinal muscle of the suspensory membrane; *x*, muscle, which on contraction drags the mouth region down toward the ciliated ridges.

(1) *Frontal cilia.* The frontal cilia are long and coarse, and are borne on a single row of large cells. They occur in a group across the inner or apical end of each cell (Fig. 6B). These cilia appear active only when stimulated by the presence, in the inhalant current, of food organisms, which they send toward the inner ends of the ridges and the mouth. When at rest they lie with the tips directed toward the outer ends of the ridges, their position of rest being at the beginning of the effective stroke. When not actively beating they yet sometimes have a slight vibratory movement.

(2) *Latero-frontal cilia.* These long and rather stout cilia are latero-frontal in position and, although differing from those of bivalves in the direction of their beat, for convenience will here be termed latero-frontal cilia. They

occur to the inner side of the frontal cilia and separated from the lateral cilia by an unciliated space. When at rest they are almost straight. They are about 20–30 μ long and 3·5 μ apart in the larva of *M. membranacea*. Only the tips appear to bend during the beat, which is slow, with the effective stroke away from the frontal and toward the lateral cilia. This direction was unexpected, but careful focusing at a magnification of 1360 diameters confirmed it. These

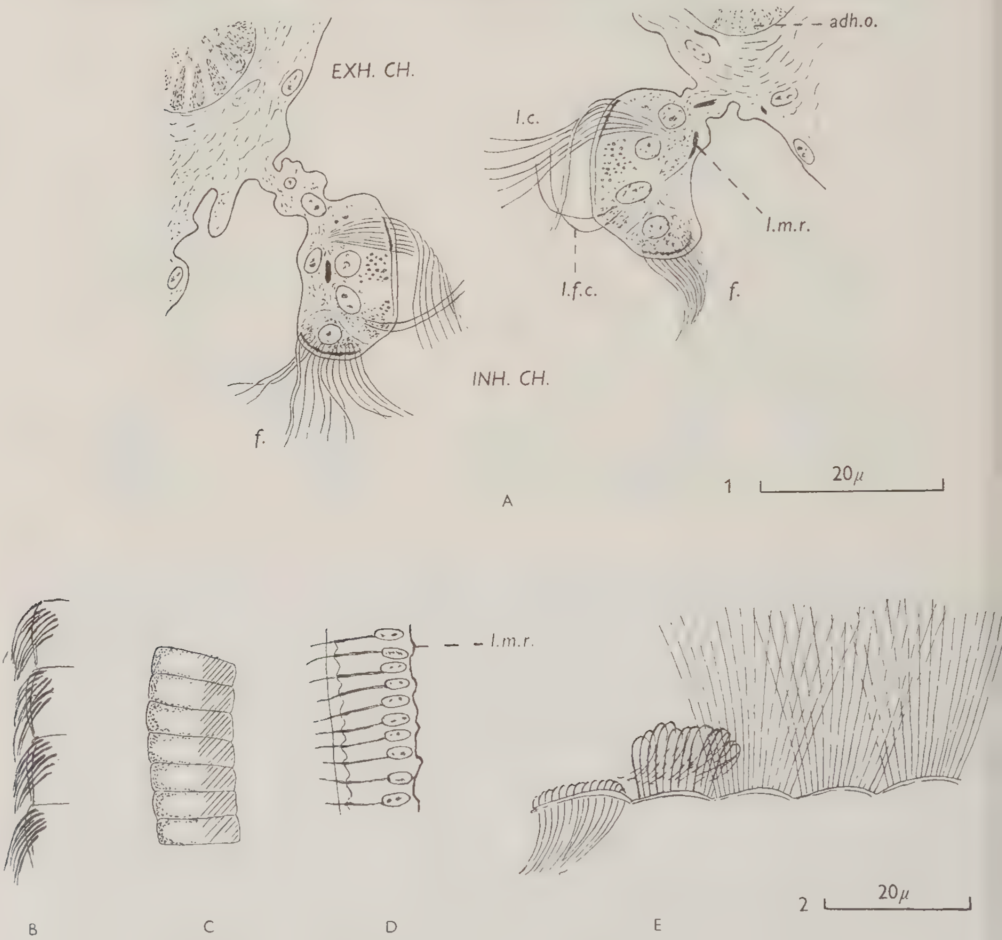

Fig. 6. *Membranipora membranacea* (L.): cyphonautes larva. A: transverse section through the ciliated ridges near their inner ends, in a specimen with well-developed adhesive organ (*adh.o.*). B: frontal cells in side view (living). C: lateral cells in surface view (living). The oblique rows of basal granules are indicated. D: latero-frontal cells in a section in a plane perpendicular to that of the beat. E: fan-shaped groups of cilia on the velum (living). *f.*, frontal cilia; *l.c.*, lateral cilia; *l.f.c.*, latero-frontal cilia; *l.m.r.*, longitudinal muscle of the ciliated ridge. *INH.CH.* and *EXH.CH.*, inhalant and exhalant chambers. A and D, Heidenhain's iron haematoxylin. A and D to scale 1; B, C and E to scale 2.

cilia probably form, to some extent, a grid between the inhalant and exhalant chambers, and, depending on movements of the suspensory membranes of the ridges, may project into the inhalant chamber, or across the aperture between the two. They are borne on a row of small, narrow, rectangular cells with their length across the length of the ridge. There is probably one cilium to a cell (Fig. 6D). The cilia appear to be arranged alternately, one being inclined at a lesser angle to the lateral surface than the next. In sections two diverging ciliary rootlets are very evident (Fig. 6A), but the fibres which proceed from these appear to form one cilium in life. Kupelwieser (1905, p. 23), however, considered that there were two cilia to a cell and that their function was sensory. He compared these ciliated cells with similar, but less developed, ciliated cells of the corona.

(3) *Lateral cilia*. The lateral ciliated cells occur in a single row on the inner surfaces of the ridges. The cells are rectangular with their length transverse to that of the ciliated ridges. The cilia are borne on the abfrontal half of the surface of the cell. The basal granules are arranged in oblique rows (seen living at a magnification of 1360 diameters) (Fig. 6C), as are those of the lateral cilia of lamellibranchs (Atkins, 1938, p. 332).

The cilia are long and beat across the length of the ridge, from the inhalant toward the exhalant chamber. They produce the main water current. The metachronal wave is that characteristic of lateral, current-producing cilia: it runs at right angles to the direction of beat, and passes up the left ridge and down the right one. The waves are laeoplectic, according to the nomenclature employed by Knight-Jones (1954, p. 504).

When at rest the cilia are wrapped across the ridge with the tips projecting beyond the frontal edge: their position of rest is at the beginning of the effective stroke.

The lateral cilia suffer intermissions which are independent of stoppage of the coronal cilia. When two or three drops of 7% magnesium chloride in tap water are added to a watch-glass in which the larvae are swimming, intermissions of the lateral cilia cease. Magnesium chloride does not appear to affect the action of the frontal, latero-frontal or mantle cilia.

The Mode of Feeding

Water, with contained food particles, is drawn into the inhalant chamber by the action of the lateral cilia on the ciliated ridges, and passes between the ridges into the exhalant chamber, leaving by the exhalant aperture (Figs. 1, 2).

When the larva is not feeding the funnel cilia are mostly inactive, as are also the frontal cilia of the ridges, and many food organisms pass through into the exhalant chamber and out through the aperture. The ciliated ridges can be separated, allowing this to occur. It is doubtful to what extent the latero-frontal cilia act as strainers, since the direction of their beat is toward the exhalant chamber, although it appears that little more than their tips bend.

When the larva is feeding the frontal cilia are active and food organisms travel up the ridges. From the inner ends of these they are conveyed over the ciliated tract of the funnel to the unciliated area in front of the oesophageal mouth. The cilia of this tract are long and powerful and some organisms in the anterior stream of the inhalant current pass directly on to this tract, without travelling up the ridges.

Organisms arriving at the oesophageal mouth are either swallowed singly or in small collections according to size, for instance *Peridinium troichoideum*, 20–30 μ in diameter, is swallowed individually, while *Chlorella stigmatophora*, 2·5–4·5 μ in diameter, is taken in small collections. These organisms were used merely to demonstrate the currents and it is not known whether the cyphonautes larva is able to digest them.

The inhalant and exhalant currents are occasionally reversed, food organisms, and at times a faecal pellet, passing from exhalant to inhalant chamber and out through the aperture. This reversal is possibly due to contraction of the shell adductor, and of muscles of the upper part of the mantle, approximating the valves and forcing water from the exhalant to the inhalant chamber. It has been observed to occur during stoppage of the lateral cilia. Reversal of the beat of the lateral cilia was not seen.

Methods resorted to by the cyphonautes whereby unwanted material is rejected are as follows. When the gut is full and no more food can be accepted, or when distasteful material arrives at the mouth, the oesophageal valve remains closed. Rejection is effected by the funnel cilia ceasing to beat—except for those over a small area in front of the mouth, which are generally active—then organisms already collected before the mouth, stream downwards, caught in the water current set up by the lateral cilia, and pass between the apical ends of the ridges into the exhalant chamber. Rejection may be assisted by the mouth region being dragged downward toward the ciliated ridges by contraction of muscles running from the body wall, over the anterior region of the stomach, to the mantle lobe about the middle of the inhalant chamber. These muscles are shown in Fig. 5A at x.

Ehrenberg (1838, p. 395 and pl. 44, fig. 2), who first described the cyphonautes, demonstrated, by means of indigo in the surrounding water, the passage of material through the vestibule to the mouth and then through the gut and out at the exhalant aperture. Claparède (1863, p. 108) also observed this current, but apparently neither of them noticed one passing between the ciliated ridges, nor did Robertson (1908). Robertson (p. 273) ascribed to the action of the cilia of the oral atrium (funnel) the current setting toward the mouth. Kupelwieser (1905, pp. 17, 26) attributed to the pyriform organ with its ciliated groove and plume the function of conveying particles into the median atrium (inhalant chamber). He (p. 26) mentioned the occasional passage of small particles from one atrium to the other, between the ciliated ridges.

THE DIGESTIVE SYSTEM

The digestive system is simple. The gut appears to be entirely ciliated, except for the valve at the entrance to the oesophagus (Fig. 7A). This valve is closed by a sphincter of striated muscle fibres. Muscle strands run between the valve and the stomach (Fig. 7B), and also between it and the mantle. The oesophagus is short: it is surrounded by a layer of strong circular muscle fibres (Fig. 7C). There appear to be no muscle fibres surrounding the stomach, intestine or rectum, but muscles pass between the gut and the mantle (Fig. 1). The stomach gradually decreases in diameter and passes imperceptibly into the intestine. There is little or no difference in the appearance of the epithelial lining of the two regions. A ventral tract of glandular or secretory cells, the contents of which stain darkly with iron haematoxylin, stretches from the anterior region of the stomach almost to the posterior end of the intestine. The tract is widest near the entrance of the oesophagus. The cells of the side walls of the stomach are particularly deep and contain many inclusions, which in life are yellow and brownish in colour. Similar inclusions are present in the less deep intestinal cells. In sections stained with iron haematoxylin the contents of the cells appear vacuolated, many of the vacuoles having faintly staining inclusions; both vacuoles and inclusions increase in size toward the bases of the cells. These cells are probably digestive in function (Fig. 7D, E).

The cilia on both sides of the constriction separating intestine from rectum are long, particularly a ring of intestinal cilia which form a long tuft or plume projecting into the intestine (Fig. 7H). From their appearance in cross-section (Fig. 7F) it would appear that the latter have a spiral or rotatory movement. They possibly function in forming faecal matter into a cord, or, together with the adjacent long rectal cilia, in passing it into the rectum. Especially common in the anterior region of the rectum are gland cells with darkly staining contents, which presumably form the transparent coat surrounding the faecal pellets. The greater length of the rectum is lined with an epithelium, the cells of which bear a coat of dense short cilia, and contain many vacuoles showing inclusions, which are possibly excretory (Fig. 7G). In sections an anal sphincter could be distinguished, but not one at the constriction between intestine and rectum, although one probably exists.

Alcian blue, after about 40 sec., does not stain the free border of the gut cells, including those of the oesophageal valve, but does stain brightly the free border of the cells of the mantle cavity or vestibule, including those of the region immediately in front of the oesophageal valve (Fig. 7A).

The exact manner in which food, which has collected in the unciliated region in front of the oesophageal valve, is taken into the oesophagus could not be followed, the movement being very rapid. There appeared to be a momentary protrusion of the valve region and sometimes contraction of muscles of the ciliated funnel. Although the gut is entirely ciliated, movement

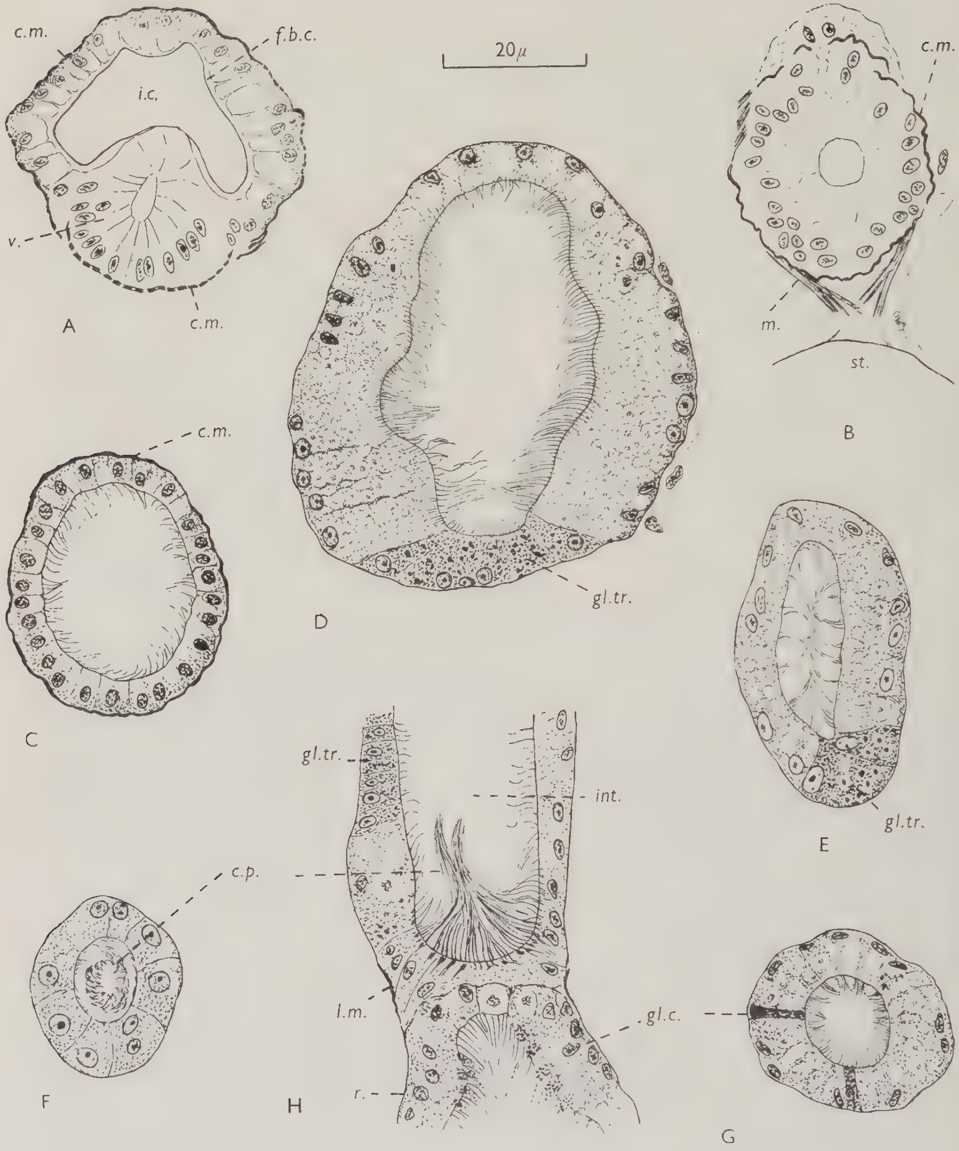

Fig. 7. *Membranipora membranacea* (L.): cyphonautes larva. A: section through the unciliated oral end of the inhalant chamber and obliquely through the valve (*v.*) leading to the oesophagus. Transverse sections of: B, the oesophageal valve; C, the oesophagus; D, the stomach; E, the upper region of the intestine; F, the lower region of the intestine, showing the ciliary plume (*c.p.*) cut across; G, the middle region of the rectum, with short cilia; H, longitudinal section through the constriction between the intestine (*int.*) and the rectum (*r.*), showing the long ciliary plume of the intestine, and the upper part of the rectum with gland cells and long cilia; *c.m.*, circular muscle fibres; *c.p.*, ciliary plume; *f.b.c.*, free border of cells which stains bright blue with alcian blue; *gl.c.*, gland cells; *gl.tr.*, glandular tract of stomach and intestine; *i.c.*, inhalant chamber; *int.*, intestine; *l.m.*, longitudinal muscle fibres; *m.*, muscle fibres passing between oesophageal valve and stomach wall; *r.*, rectum; *st.*, stomach; *v.*, oesophageal valve. Heidenhain's iron haematoxylin and alcian blue.

of food within it was rarely seen. Long cilia were visible, but appeared motionless. Exceptionally, in one larva of *Electra pilosa*, an active stirring was seen of many *Chlorella* forming the contents of the stomach.

When a cyphonautes larva is placed in water containing many *Peridinium trochoideum*, the gut becomes tightly packed with them, and faecal pellets are expelled with the peridinians almost unchanged, but bound together by mucus. It would appear that under these conditions, the peridinians are forced through the gut so rapidly that the digestive enzymes have not sufficient time in which to act. Or it may be that the cyphonautes is unable to digest peridinians, which are heavily armoured with plates, thought to consist mainly of cellulose. The gut may become so filled with *Peridinium* and *Chlorella* that no division is visible between intestine and rectum, then just before defaecation a constriction appears between the two regions. Devèze (1953) has recorded the feeding of the cyphonautes of *Electra pilosa* on micro-organisms less than 0·9 μ in diameter, but the larvae of both *E. pilosa* and *Membranipora membranacea* can certainly swallow organisms up to at least 30 μ in diameter.

THE SWIMMING OF CYPHONAUTES

The ciliated corona is the locomotive organ of the larva. The metachronal wave of these cilia passes round the corona in a clockwise direction when the larva is viewed from above, travelling without a break over the much reduced cilia of the middle region or inhalent aperture. The larva swims with the apical organ foremost: frequently it swims in a vertical position. Sometimes, while in an upright position, it will maintain itself at the same level, going around very slowly in a circle, clockwise, that is in the same direction as the metachronal wave, and swaying exceedingly slightly from side to side, the coronal cilia, meanwhile, beating with a perfect metachronal rhythm. On occasions the level was maintained so closely that the larva remained in focus for minutes on end when viewed at a magnification of 115 diameters. Suddenly the valves would contract, the coronal cilia stop beating and the larva drop: it may do this without apparent cause or when touched by another organism.

The larva probably changes direction when swimming by protruding or withdrawing part of the corona, or possibly by protruding the pyriform organ with its vibratile plume.

The work recorded in this paper forms part of a study of ciliary feeding mechanisms undertaken while holding a Leverhulme Research Fellowship.

SUMMARY

The cyphonautes larva obtains its food by maintaining a continuous current of water through the mantle cavity, which is divided into inhalant and exhalant chambers, separated by the paired ciliated ridges. The water current is

produced by lateral cilia on the ridges, on which there are also frontal, food-conveying, cilia. Food organisms travel from the inner ends of the ciliated ridges over the ciliated tract of the funnel of the inhalant chamber to the mouth.

Rejection of unwanted material is effected by the mouth remaining closed, while material in front of it is carried off in the current passing into the exhalant chamber, most of the funnel cilia meanwhile remaining inactive. Rejection is assisted on occasions by the mouth region being dragged downward toward the ciliated ridges by contraction of certain muscles in the mantle.

The alimentary system is simple. The mouth, closed by a sphincter, leads into a short oesophagus and this in turn into the stomach, which passes imperceptibly into the intestine. A constriction generally separates the intestine from the rectum. Notes are given on the histology of the gut.

Observations on swimming of the larva are given.

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THE COMPARATIVE EXTERNAL MORPHOLOGY AND REVISED TAXONOMY OF THE BRITISH SPECIES OF *IDOTEA*

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(Text-figs. 1–11)

This paper aims at describing the external morphology of seven British species of *Idotea* from the functional and developmental aspects, thus revising earlier descriptions, such as those of Collinge (1917), which were based largely on adult specimens. Limitations of earlier descriptions are apparent, but some characters can be sifted from the older literature and combined with characters emphasized in this paper to give a schematic description of the seven species.

I am grateful to Mr J. S. Colman for criticism of this work, to Dr I. Gordon for facilities at the British Museum (Natural History), and for examining the Linnaean specimens of *Oniscus marinus*; and to Mr G. M. Spooner for help in the construction of the Key.

MATERIAL AND METHODS

The crustacean genus *Idotea* belongs to the suborder Valvifera of the order Isopoda, and the seven species of the genus which are dealt with in this investigation are *I. linearis* (Pennant), *I. viridis* (Slabber),[1] *I. baltica* (Pallas),[1] *I. neglecta* G. O. Sars, *I. emarginata* (Fabricius), *I. granulosa* Rathke, and *I. pelagica* Leach.

All these species, and no others, are recorded from the Isle of Man (Moore, 1937, etc.), though the records of *I. viridis* are open to some doubt; the finding of *I. viridis* in this investigation seems to constitute the first certain record of the species in the Isle of Man (Naylor, 1955*a*). Collinge (1917), in his review of the Idoteidae, records two other species from Britain: *I. metallica* Bosc. and *I. sarsi* Collinge. *I. metallica* does not seem to be a British resident, since only single specimens have been recorded, often from amongst *Lepas* on driftwood (Tattersall, 1911), whilst *I. sarsi* is synonymous with *I. baltica*. I have examined the type specimens of *I. sarsi* in the British Museum and they cannot be distinguished from *I. baltica*.

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[1] Holthuis (1949) maintains that *I. viridis* Slabber should now be called *I. chelipes* (Pallas), and that *I. baltica* (Pallas) should now be called *I. marina* (L.). Dr Gordon has examined the Linnaean specimens of '*Oniscus marinus*', which resemble *Idotea granulosa* more than they do any other species. Neither of Holthuis's suggestions is adopted in the present paper.

I have collected all seven species, the shore species *I. granulosa* and *I. pelagica* in particular, from various localities around the Isle of Man, and all but *I. linearis* were taken in some quantity. In addition, for morphological comparison with Manx specimens, material has been obtained from other localities in the British Isles.

Material was preserved in Baker's calcium formol solution, 5% sea-water formalin, or 70% alcohol. For microscopical examination appendages were mounted directly into glycerine from freshly killed specimens; semi-permanent preparations were made in Berlèse solution of glycerine jelly.

The staining and mounting methods given by Collinge (1917, 1918) proved impracticable. They are laborious and they entail the use of Canada balsam, the high refractive index of which makes accurate study of arthropod exoskeletons very difficult.

All measurements of body length were taken down the mid-dorsal line, irrespective of the shape of the telson. The larger specimens were measured against a non-parallax scale under a low-power dissecting microscope, and the smaller specimens were measured under a monocular microscope with a micrometer eyepiece.

The terminology adopted for the major divisions of the body is that used by Hansen (1925) and Howes (1939), namely:

Cephalon: the head and the first thoracic segment fused together.

Thorax: the last seven thoracic segments.

Abdomen: strictly speaking composed of six segments, but dorsally it appears to consist of three segments, the last of which is fused to the *telson*.

GENERAL DESCRIPTION OF *IDOTEA*

Size and shape

The length of a juvenile newly emerged from the brood-pouch varies from 1 to 3 mm, according to the species. Males of the shortest species, *I. pelagica*, are recognizable at about 4 mm, and they range to about 12 mm. On the other hand, males of a larger species, *I. emarginata*, are identifiable at about 7 mm in some cases, and they range to about 30 mm in length. Within any one species, the size range of females is less than that of the males, and the female of a copulating pair is always smaller than its mate.

Shape varies from the long, filiform *I. linearis*, to the small, robust form of *I. pelagica*, and in all cases the ovigerous female is relatively broader than the male of the same species.

The Front Cephalic Appendages

Antennules (Fig. 1)

The antennules are the most anterior of the appendages. Each has a three-jointed basal part and a flagellum consisting of one joint, the latter bearing on its antero-medial surface a number of aesthetascs. Each aesthetasc consists of

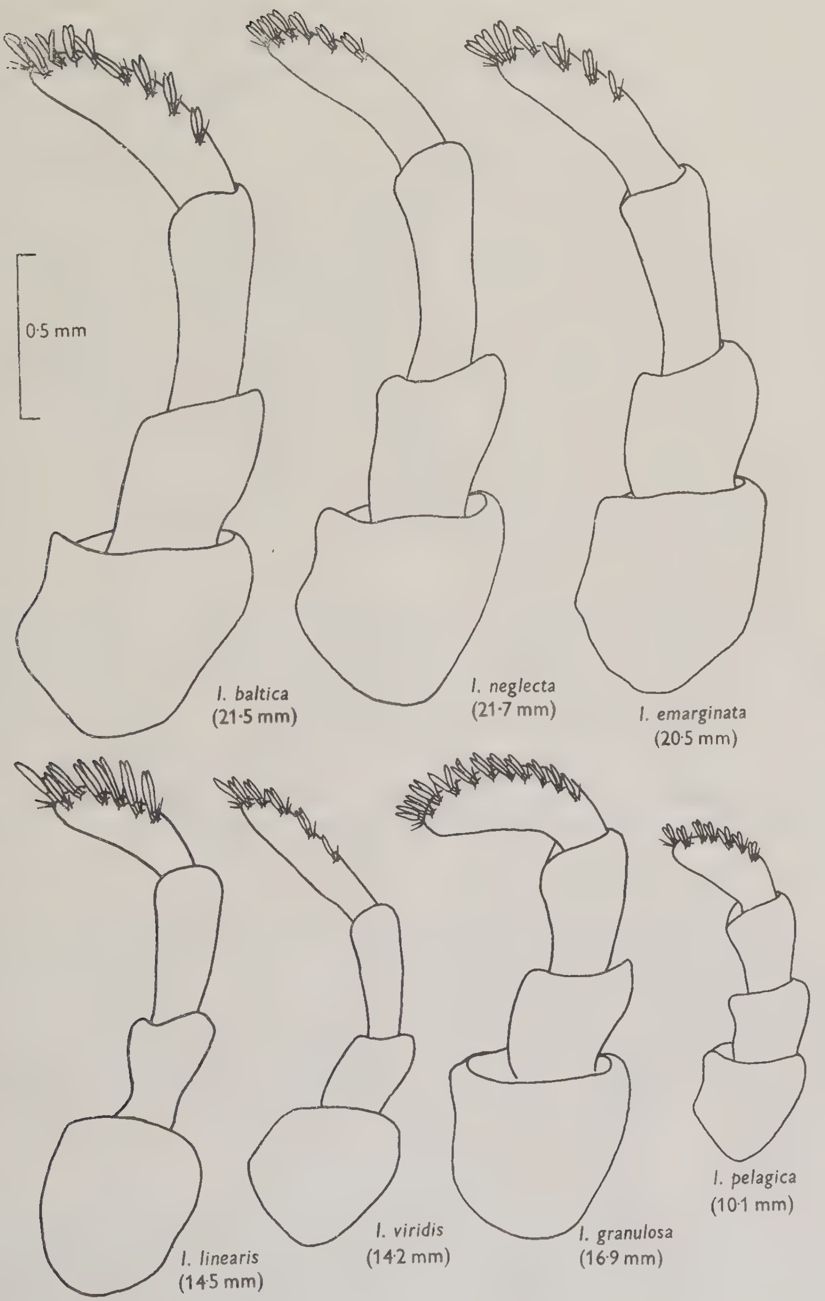

0·5 mm
I. baltica
(21·5 mm)
I. neglecta
(21·7 mm)
I. emarginata
(20·5 mm)
I. linearis
(14·5 mm)
I. viridis
(14·2 mm)
I. granulosa
(16·9 mm)
I. pelagica
(10·1 mm)

Fig. 1. The right antennule of adult *Idotea*, seen from below.

a fairly strongly chitinized stalk with a distal portion apparently opening at its tip by a pore. Nothing seems to be known about their function except that they are generally assumed to be 'sensory'. Howes (1939) first noticed and described the aesthetascs in *I. viridis*, and the fact that they were not previously observed is probably related to the methods of mounting and preservation; they are well seen in freshly killed material mounted in glycerine.

As can be seen from Fig. 1, the arrangement and number of aesthetascs may vary from species to species in adult males. The number also varies with age, since newly released juveniles have only a single aesthetasc (in *I. linearis* and *I. viridis*) or a pair of aesthetascs (in other species described) situated at the tip of the antennule. As growth proceeds others are acquired, at first singly, and later usually in pairs, away from the tip. Even though these subsequent aesthetascs may be acquired singly they are always eventually arranged in pairs, whatever the species; any single aesthetasc, other than the terminal one of *I. linearis* and *I. viridis*, is added to at the next moult.

In addition to the arrangement, the *rate* of addition of aesthetascs is often characteristic for each species, as will be shown later (p. 478).

Antennae (Fig. 2)

The antennae in all species have a peduncle consisting of five segments, and a terminal flagellum with a varying number of joints. In newly released juveniles of each species the flagellum has two segments, and subsequent segments are budded off from the basal segment of the original two. Once a segment has been formed, provided it is not a replacement due to breakage, its shape is fairly characteristic for each species and is reflected in the shape of the antennal flagellum as a whole. In *I. pelagica* and *I. granulosa* the segments are stout and the flagellum is correspondingly short, whereas in *I. baltica*, *I. viridis* and *I. linearis* the segments are much longer than they are broad and the flagellum itself is long and slender.

The rate of addition of segments relative to the absolute length of the body is much the same for each species, so that an adult *I. pelagica*, 11 mm in length, has about the same number of segments in the antennal flagellum as an immature specimen, 11 mm in length, of a larger species.

An additional feature in *I. pelagica* and *I. granulosa* is that adult males have thick pads of small setae along the segments of the flagellum of the antennae; their function is not understood, but they are lacking from the other species examined.

The Mouthparts

Fig. 3 illustrates a typical set of mouthparts of *Idotea*, actually of *I. emarginata*. They consist of the paired mandibles, the median paragnath, the paired maxillules and maxillae, and the paired maxillipedes. The strongly chitinized, plate-like labrum fits in front of the mandibles and projects downwards and backwards; it is not figured.

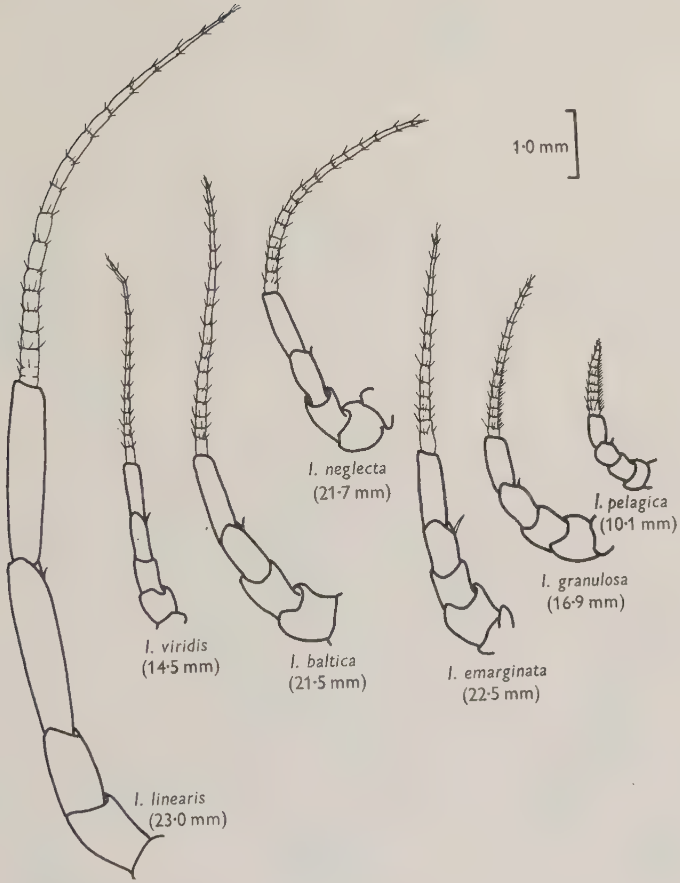
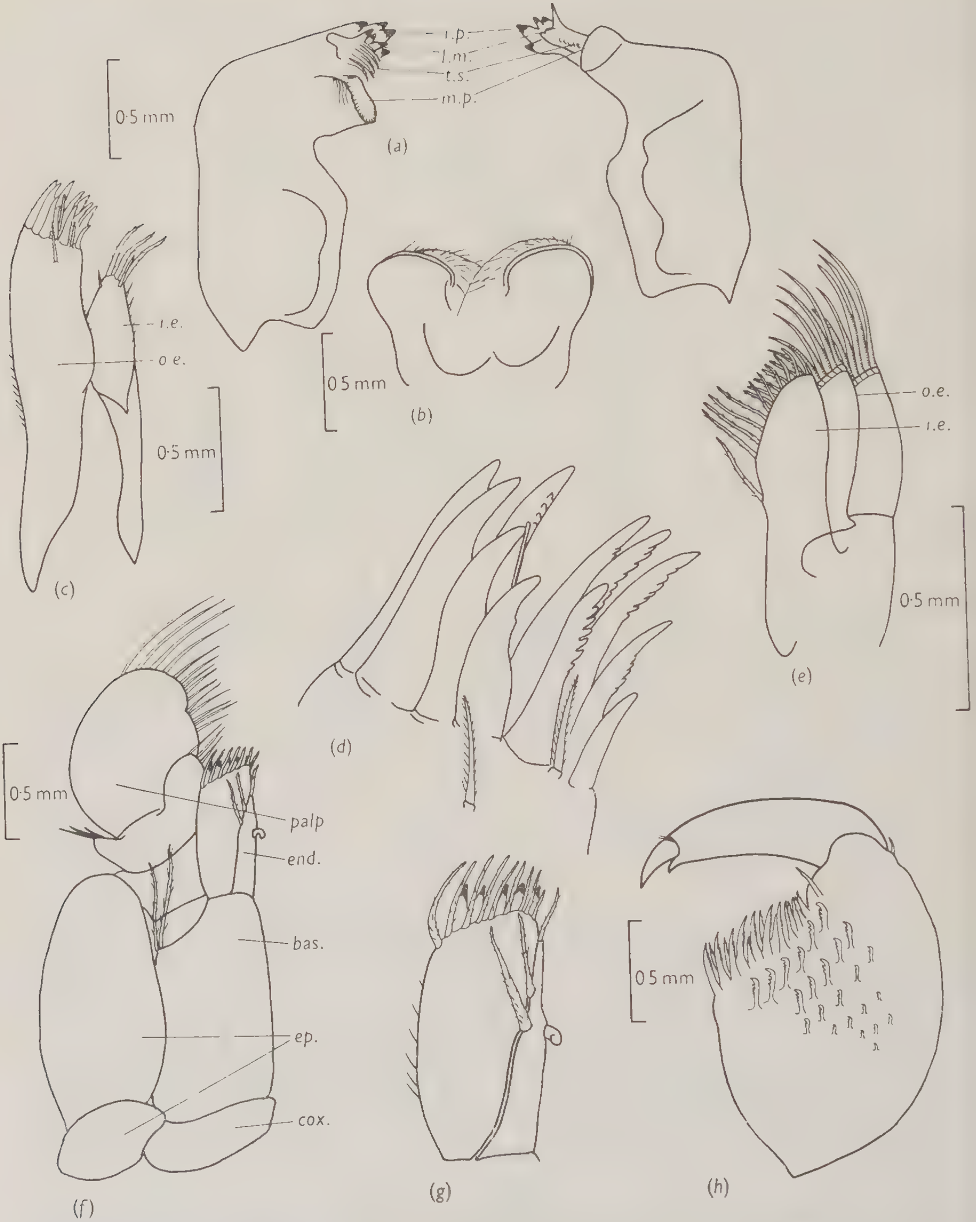

1·0 mm
I. linearis
(23·0 mm)
I. viridis
(14·5 mm)
I. baltica
(21·5 mm)
I. neglecta
(21·7 mm)
I. emarginata
(22·5 mm)
I. granulosa
(16·9 mm)
I. pelagica
(10·1 mm)

Fig. 2. The right antenna of adult *Idotea*, seen from above.

Mandibles (Fig. 3*a*)

It has not previously been noted that the mandibles of *Idotea* are asymmetrical, though the feature is probably common in the Malacostraca as a whole (Calman, 1909). Each mandible is L-shaped; one arm has strong muscular attachments and lies horizontally, parallel to the sides of the head, whilst the other arm points downwards and inwards, meeting its fellow medially on the ventral side of the head. Ventrally placed on each mandible is a strongly chitinized incisor process, and above this is the lacinia mobilis, which is more strongly chitinized on the left mandible than on the right. When the mandibles are closely apposed, the incisor process of the right appendage fits between the lacinia and the incisor process of the left side.


i.p.
l.m.
t.s.
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0·5 mm
(a)
(b)
0·5 mm
i.e.
o.e.
0·5 mm
(c)
o.e.
i.e.
0·5 mm
(e)
(d)
0·5 mm
palp
end.
bas.
ep.
cox.
(f)
(g)
0·5 mm
(h)

Fig. 3. The mouthparts of an adult *I. emarginata*, 20·5 mm in length: (*a*) *mandibles*, from above (*i.p.*, incisor process; *l.m.*, lacinia mobilis; *t.s.*, toothed spines; *m.p.*, molar process); (*b*) paragnath; (*c*) right *maxillule*, from below (*i.e.*, inner endite; *o.e.*, outer endite); (*d*) tip of outer endite of maxillule; (*e*) right *maxilla*, from above (*o.e.*, outer endite; *i.e.*, inner endite); (*f*) left *maxillipede*, from above (*end.*, endite; *bas.*, basipodite; *ep.*, epipodites; *cox.*, coxopodite); (*g*) endopodite of maxillipede; (*h*) terminal joint of *1st leg*.

Above the lacinia is a row of upwardly curving toothed spines, and above the spines is the molar process. The molar processes of the two mandibles are arranged differently; the left molar process faces upwards, whilst that of the right side faces downwards.

The mandibles lack any trace of a palp.

Paragnath (Fig. 3*b*)

The ventral mouth opening, bordered anteriorly by the labrum and laterally by the mandibles, is bordered posteriorly by the median, bilobed paragnath. Between the left and right lobes of the paragnath is a median groove into which project brush setae of the other appendages (see below). The median surfaces of the paragnath are covered with curved spines, which project forwards and upwards into the oral cavity.

Maxillules (Fig. 3*c*)

Each maxillule has two endites, the large outer endite and the smaller inner endite, each having fairly strong muscular attachments. The outer endite has at its tip twelve chitinized spines and two flexible flattened setae fringed with small stiff bristles (Fig. 3*d*). *This arrangement of spines and blades is the same for each species, throughout the whole of the life cycle.*

The maxillules, like the maxillae and the maxillipedes, are attached posteriorly and project forwards below the paragnath. The ventral spines of the outer endite, which are used in scraping the food material, appear spatulate and are mostly without teeth; one of this group is a rod-like structure. The upper (inner) spines, except for the small median one, are usually strongly toothed; these spines serve to push food into the mouth (Naylor, 1955*b*).

The inner endite again has a constant number of processes at its tip. They consist of four brush setae (three long and one short), with a small spine at the bases of the setae.

Maxillae (Fig. 3*e*)

These are more fragile, flattened structures, much less chitinized than the maxillules. Each again has two endites, the outer endite itself being bilobed. Both lobes of the outer endite have a row of combing setae along their anterior edges and, as can be seen in Fig. 3*e*, the outer setae show an increase in size.

The inner endite lies above and medial to the outer endite. It has two rows (upper and lower) of small brush setae, merging to smoother spines laterally, and there are, placed medially, two to four large brush setae analogous to those on the inner lobe of the maxillule.

Maxillipedes (Fig. 3*f*)

The maxillipedes are broad, flattened appendages covering the other mouthparts ventrally. Arising laterally from the coxa are two epipodites, and arising medially is the basis. Articulating in front of the basis are the medial endopodite and the distal four-jointed palp, the outer lobe of which bears long smooth spines.

The endopodite (Fig. 3*g*) is turned vertically upwards along its medial edge and is thus L-shaped in cross-section. The appendage of each side has a single coupling hook situated on the vertical flange, and these interlock to make the maxillipedes, functionally, a single structure. The L-shape of the endopodite was first described by Howes (1939) for *I. viridis*, but it is common to all the seven species.

Along the upper edge of the endopodite, and extending forwards and laterally across the anterior border, is a row of brush setae, again comparable with those of the preceding two pairs of appendages. Beneath the brush setae is a row of heavily chitinized, smooth, scraping spines which have a curious double chisel edge at their tip (Naylor, 1955*b*).

A feature not mentioned by Collinge (1917) is to be found on the maxillipede of the adult female. The appendage as described so far is typical of all specimens, but in females with a brood pouch there is, in addition, on the inner posterior border of the maxillipede, a small lappet fringed with setae (Fig. 4*e*). This projects into the brood pouch, since the first oostegite covers the base of the maxillipede, and it appears that movements of the maxillipede cause the lappet to create a current of water over the developing eggs in the brood pouch. Ovigerous females, otherwise at rest on weed, and certainly not feeding, are often seen to raise and lower their maxillipedes with some regularity.

Calman (1909), not specifically referring to *Idotea*, states that 'in ovigerous females of...certain genera of the Valvifera the coxopodite bears a small lappet, fringed with setae, projecting backwards into the marsupium'. By virtue of the position of this structure, and its occurrence in the adult female, Calman suggests that the lappet may be homologous with the oostegites of the first five thoracic segments.

The Appendages of the Thorax

Legs

The thorax is composed of seven segments, and in adult specimens each segment bears a pair of walking legs. Newly released young have only six pairs of legs, the last segment being small and devoid of appendages. After two moults, in *I. emarginata* and probably in other species as well, the seventh segment acquires the first signs of a pair of legs in the form of a pair of small rudiments projecting forwards. Only after a third moult does the animal acquire its full complement of legs.

The first pair of limbs are short and each has a palmate distal joint (dactylopodite), which bears rows of comb spines on its inner surface (Fig. 3*h*). This surface is held beneath the mouthparts during feeding, and it aids in keeping them free of debris.

On the more posterior segments the legs become longer and more slender, being particularly so on the last three thoracic segments. None of the last six pairs of legs are used in feeding, but some are modified in the adult male and female. The second pair of legs of males of all species (and all succeeding legs in *I. linearis* and *I. pelagica*) bear thick pads of fine setae on their inner surfaces (Fig. 4*c*). This pair of legs is used by the male in clasping the female in the

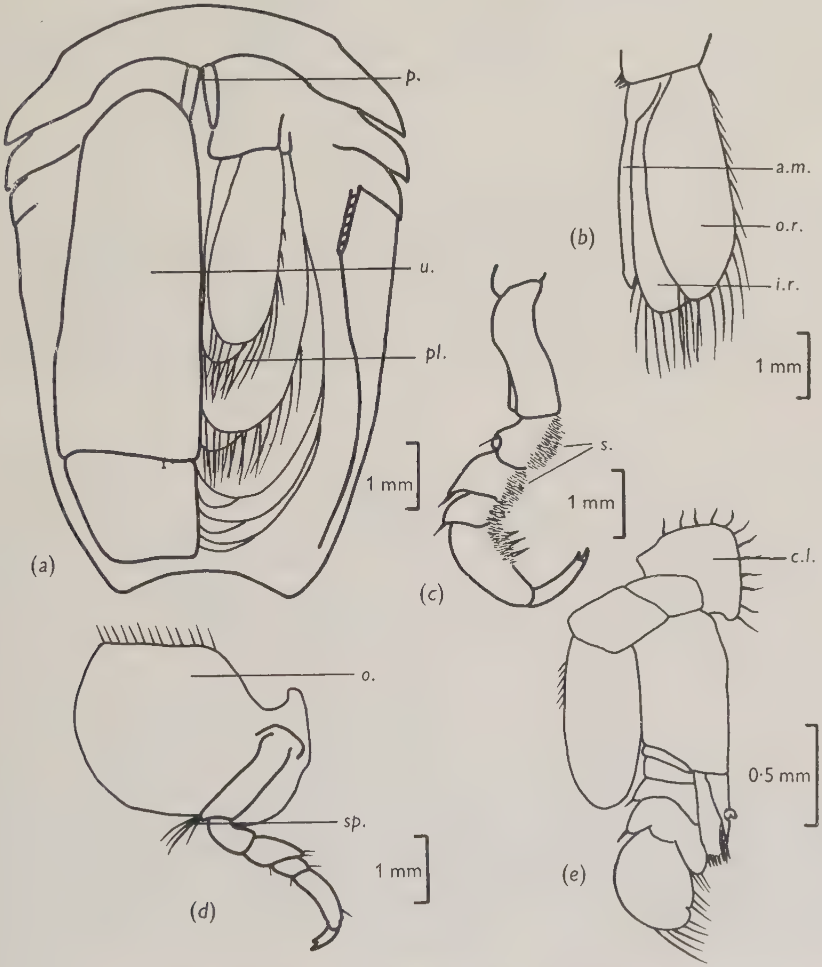

Fig. 4. Secondary sexual characters of *Idotea emarginata*: (*a*) *abdomen of male*, from below, showing penes (*p.*) (*u.*, uropod; *pl.*, pleopod); (*b*) *appendix masculinus* (*a.m.*) on the second pleopod of the male (*o.r.*, outer ramus of pleopod; *i.r.*, inner ramus); (*c*) *2nd leg of adult male*, showing pads of finesetae (*s.*); (*d*) *4th leg of adult female*, with spines (*sp.*) and oostegite (*o.*); (*e*) *maxillipede* of adult female, showing lappet (*c.l.*).

periods of pairing prior to copulation, when it would appear that the pads of setae give a good grip on the smooth surface of the female.

In adult females the first five or six legs acquire spines which support the oostegites in their task of forming a brood pouch. The legs flex inwards beneath the body and a 'knee' joint is formed between the ischiopodite and the meropodite, the two basal segments. It is from the most distal and inner edge of the basal segment, the ischiopodite, that a number of spines fan out beneath the oostegites (Fig. 4*d*).

Coxal Plates (Fig. 11)

The shape of the coxal plates of the thoracic limbs is often regarded as important in separating species of *Idotea*. The extent to which the plates project from beneath the dorsal plates of the body is fairly characteristic for each species, though in some species the shape is very similar. The coxal plates of a slender species like *I. viridis* project to a less extent than those of a more robust species.

Oostegites (Fig. 5)

Oostegites, membranous structures enclosing the brood pouch, arise from the inner parts of the coxae of the first five pairs of legs of adult females. Collinge (1917) states that they are to be found only on the third and three succeeding segments, but Kjennerud's (1950) description of the oostegites of *I. neglecta* from Norway agrees with the present description for all species dealt with in this paper.

Fig. 5 shows the oostegites of the right side of an adult female *I. emarginata* seen from above. The members of the first pair of oostegites are so shaped as to cover both the first thoracic segment and the bases of the maxillipedes, without impeding the movement of the cephalon on the thorax. Hooked processes on the members of the first pair of oostegites serve to link the two together. The remaining ones are saucer-shaped, and the last two pairs bear long spines on their inner edges. The spines presumably serve to retain the eggs in the brood pouch, whilst at the same time permitting the flow of water created by the coxal lappet of the maxillipede.

The External Genitalia

The male genital ducts open at the tips of the paired penes, the latter arising from the first abdominal segment and projecting back towards the bases of the pleopods (Fig. 4*a*).

The female genital ducts open on the fifth thoracic segment.

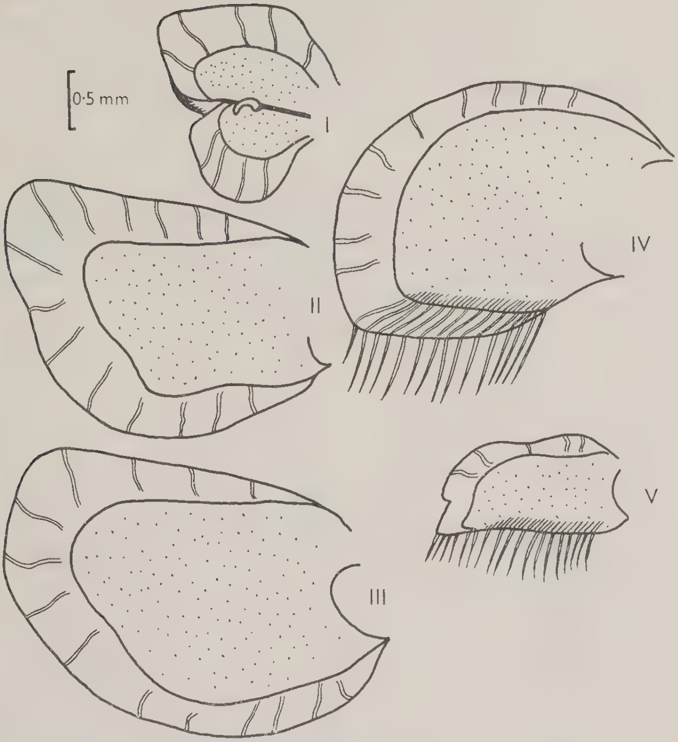

Fig. 5. The right oostegites of an adult female *I. emarginata*, from legs I–V, seen from above.

The Appendages of the Abdomen

Idotea illustrates the typical malacostracan arrangement of six pairs of abdominal limbs, namely, five pairs of pleopods and one pair of uropods. Fusion of the segments themselves, however, is such that only three are distinguishable, the last of these being incompletely fused with the telson.

Pleopods

The pleopods consist of five pairs of bilobed, lamellar structures, arising close together behind the last thoracic segment and projecting backwards beneath the abdomen (Fig. 4*a*). The current of water created by movement of these appendages serves for both respiration and swimming.

The first two pairs of pleopods possess long setae fringed with fine hairs, but such setae are lacking from the other pleopods. The inner ramus of the second pleopod of adult males bears a stylet, or appendix masculinus (Fig. 4*b*), a paired structure which serves to transfer sperm from the penes to the oviducts. The relative size of the appendix masculinus decreases as the male matures (Fig. 6) and fully mature males have an appendix masculinus of fairly characteristic shape for each species (Fig. 7).

The anus opens posterior to the pleopods, and fāeces are shed into the current of water created by the pleopods.

Uropods

A pair of large, flattened uropods are hinged laterally and folded medially to cover the pleopods when the animal is at rest (Fig. 4*a*). An endopodite articulates posteriorly with the main part of the uropod, and it has a plumose seta arising from its base and projecting backwards.

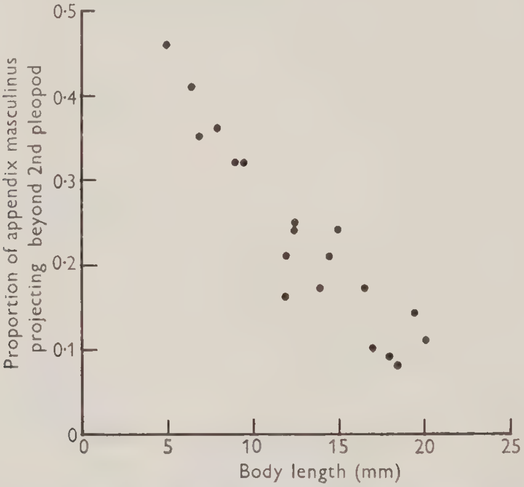

Fig. 6. The proportion of the appendix masculinus which projects beyond the second pleopod, plotted against the body length in mm, for *I. granulosa.*

The Telson

All species begin life with a similar, uniformly rounded telson, but as growth proceeds each species acquires a characteristic telson shape. Fig. 8 illustrates the shape of the telson of specimens of various sizes of all species studied; it shows the close similarity of telson shape in young forms, even of such species as *I. baltica* and *I. emarginata.*

THE TAXONOMIC VALUE OF VARIOUS STRUCTURES

Numbers of Aesthetascs and Antennal Segments

Howes (1939) noted that the number of aesthetascs and antennal segments in *I. viridis* increased as the animal increased in length. With sufficient data he was able to divide the growth of *I. viridis* into a series of clear-cut growth stages (instars), each growth stage having a characteristic number of antennal segments and aesthetascs, and having a characteristic size-range. The characters of one stage changed to those of the succeeding one after a moult.

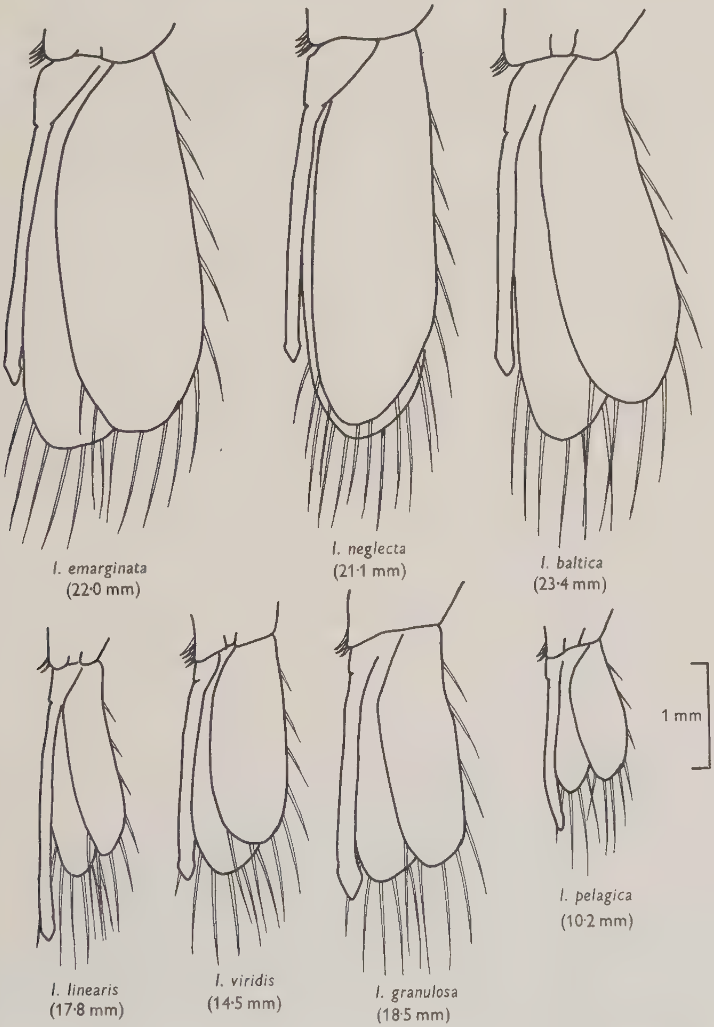
I. emarginata
(22·0 mm)
I. neglecta
(21·1 mm)
I. baltica
(23·4 mm)
I. linearis
(17·8 mm)
I. viridis
(14·5 mm)
I. granulosa
(18·5 mm)
I. pelagica
(10·2 mm)
1 mm

Fig. 7. The second pleopod of male *Idotea*, showing the appendix masculinus in each case.

Kjennerud (1950) treated *I. neglecta* in the same way and obtained a similar series of instars. Obvious differences between similar stages of the two species led Kjennerud (p. 15) to express the view that 'very little is known of the first stages of development in *Idotea* spp., although it is possible that the relation between the size of the animal, the joints on the flagellum of the antenna

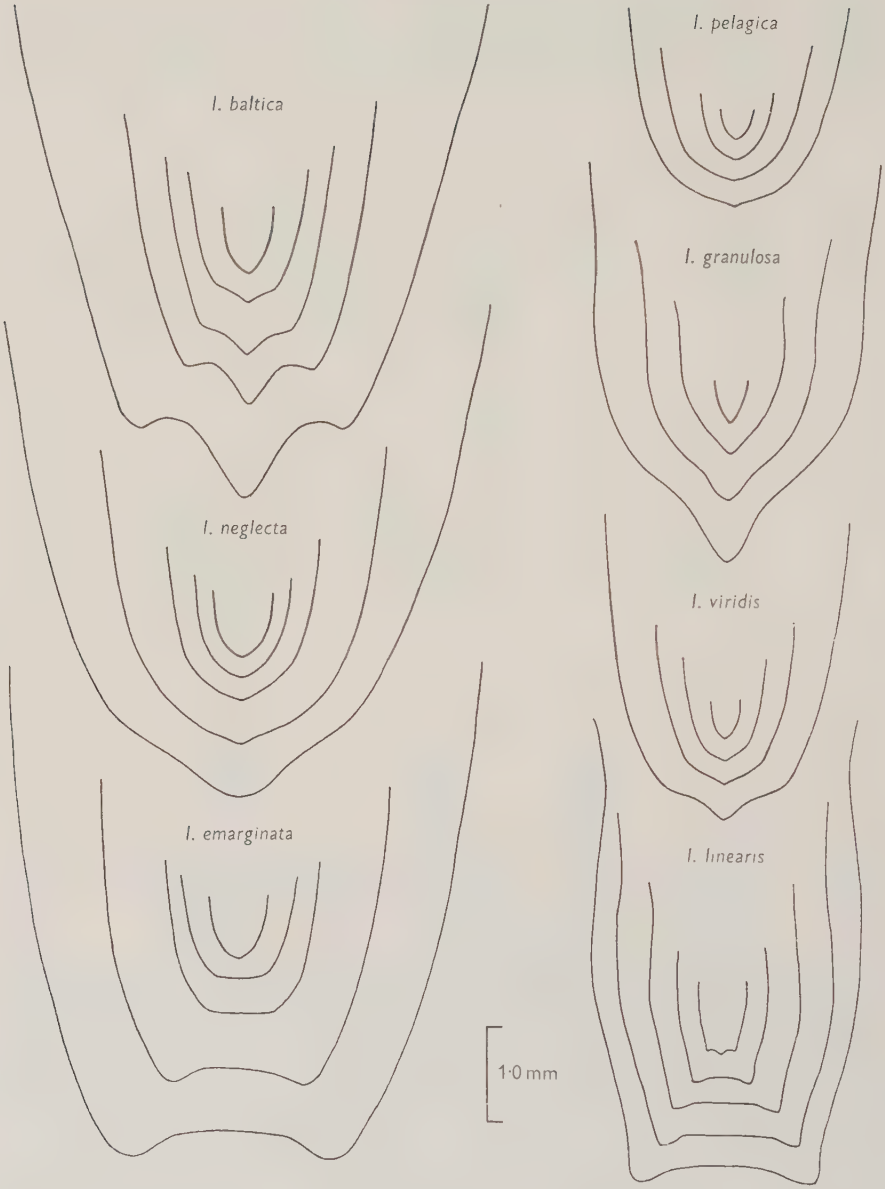
I. baltica
I. neglecta
I. emarginata
I. pelagica
I. granulosa
I. viridis
I. linearis
1·0 mm

Fig. 8. The development of the telson of seven species of *Idotea*. The body lengths of the specimens under each species were as follows: *I. baltica* (2·2, 5·4, 8·5, 12·0 and 20·0 mm); *I. neglecta* (2·7, 4·3, 6·6, 13·8, and 19·0 mm); *I. emarginata* (2·2, 4·6, 6·8, 12·8 and 19·5 mm); *I. pelagica* (1·7, 3·7, 7·2 and 10·2 mm); *I. granulosa* (1·9, 6·3, 10·8, and 16·2 mm); *I. viridis* (1·7, 4·6, 8·0 and 14·0 mm); *I. linearis* (3·5, 6·3, 11·0, 16·2 and 22·9 mm).

and the number of sense rods on the distal joint of the antennula will prove to be so characteristic for each species that one can distinguish not only between various *Idotea* species, but also between the different instars within each species'.

The principle has been applied to Manx specimens of *I. emarginata*, but it was found difficult to divide the growth of this species into discrete instars (Naylor, 1955*c*). Greater variation than was found by Kjennerud, both in the number of aesthetascs and in the number of joints in the antennal flagellum, limits the general application of the method; this applies not only to *I. emarginata* but to Manx and other British specimens of *I. neglecta*, *I. viridis*, and other species as well. In addition, as has been stated earlier (p. 470), the rate of addition of antennal segments with increasing body length is much the same for each species, except *I. viridis*.

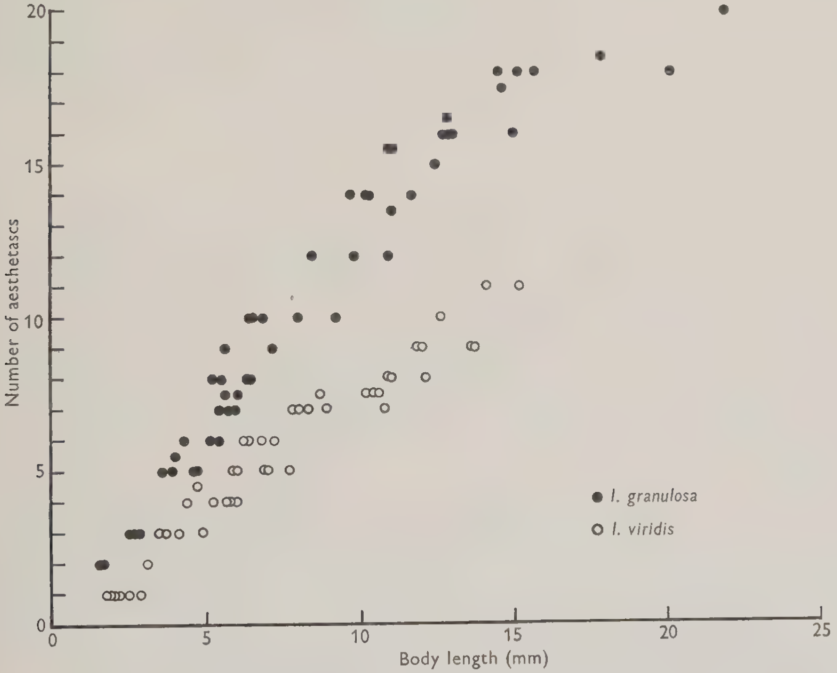

Fig. 9. Individual plots of the number of aesthetascs against body length, for fifty specimens each of *I. viridis* and *I. granulosa.*

Even, however, if division into instars and its use in taxonomy is subject to limitation, there is some taxonomic value in plotting the number of aesthetascs against the length of the body of the idoteid, irrespective of instars; the two species *I. viridis* and *I. granulosa*, which are often confused, may be completely separated by this character. Fig. 9 shows individual plottings of the number of aesthetascs against body length, for specimens of *I. viridis* and *I. granulosa.*

It will be seen that the two species hardly overlap, and they may be separated at all sizes; except in large specimens *I. granulosa* has more aesthetascs than its body length expressed in millimetres, whilst *I. viridis* generally has fewer.

The Form of the Antennule

The arrangement of the aesthetascs, rather than their number, is probably of more value in taxonomy. Reference to Fig. 1 shows that *I. viridis* and *I. linearis* may be separated from all other species by their single aesthetasc at the tip of the antennule; every other species has a pair.

As to Howes's (1939) objection that the antennules of marine species are liable to colonization by Protozoa, the aesthetascs are readily distinguishable from any such parasites as might occur, and the Protozoa are rarely in such numbers as to obscure the aesthetascs completely.

The Form of the Antenna

Collinge (1917) comments on the shape of the antenna for each species, and he emphasizes that the shape of the tip of the antenna is characteristic for each species throughout the whole of its life. In general, the shape of a segment of the flagellum remains the same, once it has been formed (p. 470). Normally there are never less than two segments on the flagellum of an *Idotea*, however small, and, since new segments are budded off from the basal segment, the terminal segment should retain its shape throughout life. If, however, the antenna is broken, then the newly formed tip may differ in shape from the original.

The antennal flagellum is probably of more use in taxonomy if it is considered as a whole. Fig. 10 shows the curves for mean length of the antennal flagellum plotted against body length for fifty specimens of each species studied (only twenty-six *I. linearis*). There is some overlapping, particularly in the young stages, but, since the relationship is roughly linear for each species the measurements might serve to help in specific identification.

The variation in the shape of the antennal flagellum, from species to species, is best understood when the mode of life of each species is taken into consideration. Species living in situations exposed to wave action have short, stout appendages, whilst those living in sheltered situations have more fragile appendages (Naylor, 1955*a*).

The Maxillule

Collinge (1917) figures and describes the tip of the outer endite of the maxillule for each species, and he states that there is a characteristic number of maxillulary spines for each species. Using these data in a key, Nierstrasz & Steckhoven (1930) separate five species of *Idotea* on the number and degree of toothedness of the spines on the maxillule. More recently, Howes's (1939) description of the spines in *I. viridis* and Kjennerud's (1950) description of those for

I. neglecta both differ from the data given by Collinge for the same species.

The present descriptions again differ, in that every species has the same number of spines on the maxillule, namely, twelve (p. 473). This has been verified in specimens of all species from many British localities, and Kjennerud's description for *I. neglecta* from Norway agrees with British specimens except that it does not include the two blade-like structures fringed with setae (Fig. 3*d*). Howes's description for *I. viridis* omits the most slender of the spines, as well as the blade-like structures.

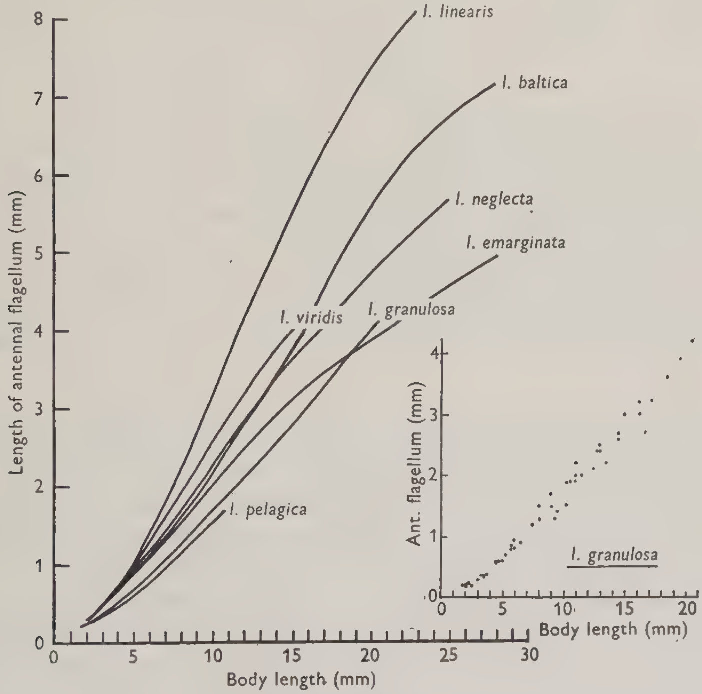

Fig. 10. Mean curves, showing the length of antennal flagellum plotted against body length, for seven species of *Idotea*. Inset shows individual plots for fifty specimens of *I. granulosa*.

The degree of toothedness of the spines varies from species to species, and sometimes throughout the life of each species. Broadly speaking though, the ventral spines, used in scraping, are less toothed than the dorsal ones, which are used in pushing food masses upwards between the mandibles (Naylor, 1955*b*).

In conclusion, it can be said that observations on the maxillules of Manx and other British specimens of *Idotea* do not conform with descriptions given by Collinge (1917), and the key for identification of *Idotea* given by Nierstrasz & Steckhoven (1930) appears to be partly invalidated.

The Coxal Plates

A taxonomic feature often emphasized, and particularly so by Dollfus (1894–5), was the shape of the coxal plates. There is some variation in the form of the plates as growth proceeds, as has been described for *I. viridis* by Howes (1939), but the feature still seems to be of some importance in separating the species (p. 476).

The Appendix Masculinus of the Second Pleopod

Though Sars (1899) regarded this structure as important, and figured it for each species he described, Collinge (1917) fails to mention it in his review. Kjennerud (1950) found the appendix masculinus of *I. neglecta* to be much longer, relative to the length of the pleopod, than allowed for by Sars, and Kjennerud quotes Tattersall (1906) as agreeing with this objection. Manx specimens of *I. neglecta* again show an appendix masculinus longer than that described by Sars, but still further modifications of all Sars's descriptions are necessary since it can be shown, in British specimens of all species, that once the appendix masculinus has attained its adult shape it increases in length at a slower rate than the inner ramus of the second pleopod (p. 478).

The length of the appendix masculinus is therefore of use in separating the species (Fig. 7), provided the length of the specimen is taken into consideration.

The Telson

Conflicting reports are to be found as to the constancy of shape of the telson of each species, throughout its life-history. Collinge (1917) found few variations in the shape of the telson, and Sars (1899, p. 81) states that he found the terminal segment of the body of *I. baltica* 'to be pretty constant even in very young forms'. Tattersall (1906), on the other hand, found some difficulty in distinguishing between young forms of different species because of the similarity in shape of the telson. Bate & Westwood (1868, p. 381) describe for *I. tricuspidata*, 'variations in the form of the terminal segment of the body, with length and size of respective specimens', but it should be noted, as Kjennerud points out, that the telson shapes illustrated for *I. tricuspidata* by Bate & Westwood probably belong to three or four different species.

Howes (1939) discussed these points of view in the light of his own work on *I. viridis*, in which changes in shape of the telson did take place as the animal aged, and he found (p. 307) 'reason to believe that changes in shape of the abdomen occur during the growth of *I. baltica* as they do in *I. viridis*'.

The present investigation reveals the fact that changes take place in the shape of the telson of all species, each beginning life with a more or less uniformly rounded telson and only acquiring the typical adult shape later in life (Fig. 8). Thus, as with many features, though the adult telson shape is characteristic for each species, the juvenile telson shapes are by no means distinct from one another. Sars's figures of adult telsons are very good for the species which he deals with, but the figures given by Collinge are sometimes inadequate.

An additional factor: Habitat

The ecology of *Idotea* is discussed elsewhere (Naylor, 1955*a*), and it is shown that each species has a fairly characteristic habitat. *I. pelagica* and *I. granulosa* are resident intertidal species, *I. viridis* is a brackish water form, and *I. emarginata*, *I. neglecta*, *I. baltica* and *I. linearis* are chiefly sublittoral. If therefore the habitat of a specimen is known it could well aid in identifying that specimen.

DISCUSSION

Any attempt to classify organisms must seek differences which are readily measured, and which are constant for similar stages of the organisms concerned. Thus, in the case of *Idotea*, which attains adult form only gradually, rather than by a distinct metamorphosis, any differences must ideally persist over the whole development of the animal. This factor appears to limit many of the earlier descriptions; the full extent of the life-cycle seems not to have been covered in the descriptions such as those by Collinge (1917), and some cases of apparent misidentification are to be found in subsequent literature.

It is with this in mind that Howes (1939, p. 307) ends his discussion of the growth of *I. viridis* with the statement that 'only the breadth/length ratio of the cephalon and the number of spines on the outer lobe of the maxillule emerge as constant throughout the whole period (of growth)'. With regard to these characters, it has been shown above (pp. 473 and 483) that the number of spines on the maxillule is useless as a taxonomic character because all species have the same number; and reference to the species-by-species descriptions given below will show the close similarity of the cephalon measurements. Therefore, of the characters studied by Howes, none would appear to be of certain value in taxonomy.

The general study of growth of all species does, however, reveal some differences of value in separating species. What is more, in the light of Huxley's (1932) emphasis of the importance of allometry in taxonomy, rates of change, such as the rate of addition of aesthetascs on the antennule, might well be used in separating some species.

The problem, therefore, in giving adequate taxonomic descriptions of species, is to fit the recent principles of experimental taxonomy with their element of 'change', into the older concept of the more 'static' nature of the species (see Gilmour, 1952). This applies even more if local differences are found within an individual species; *I. viridis*, for example, may mature at different instars in different localities (Howes, 1939), and this may well be true of other species as well. More work is clearly needed to determine any geographical variation within any one species.

An attempt has been made to embody the characters of later developmental stages, as well as those of adults, in the following key.

KEY TO BRITISH *IDOTEA*

(The smallest specimens, up to about 6 mm, are disregarded.)

1. Telson with apical border straight or concave.
   - (*a*) Body elongate, narrow: mesosome segments 2–7 less than twice as wide as long. Antenna > ½ body length. Coxal plates very small, not reaching posterior border of any segment (Fig. 11). Telson with lateral corners acutely angled, and with a smaller median spine in youngest specimens. Colour brown-green, with white or darker markings. Mainly sublittoral, in fully marine conditions. **I. linearis** (L.)
   - (*b*) Body less elongate: mesosome segments 2–7 twice as wide as long, or more. Antenna < ½ body length. Coxal plates 3–7, at least, reaching posterior border of segment.
     - (i) Telson with apical border concave and angulate at sides (adult) or straight with rounded corners (juvenile), the sides somewhat rounded (Fig. 8). Coxal plates 5 and 6 not sharply angled (i.e. angle > 45°). In shallow coastal waters; mainly sublittoral. **I. emarginata** (Fab.)
     - (ii) Telson with apical border straight, with rounded corners (adult) or widely rounded corners (juvenile); the sides straight. Coxal plates 5–7 very sharply produced, making angles of less than 45°. An infrequent, off-shore species. **I. metallica** Bosc.
2. Telson with apical border produced and more or less angulate in centre.
   - (*a*) Telson apical border tridentate or nearly so; median process acute and well protruding. Green or brown, often with pattern of spots and lines. Marine, chiefly sublittoral, in shallow water. **I. baltica** (Pallas)

     (N.B. Large specimens of *I. viridis* may have angled shoulders to the telson, but this brackish water species differs structurally from *I. baltica* by having a single aesthetasc at the tip of the antennule, instead of a pair, and coxal plates 2, 3 and 4 do not reach the posterior border of the segment as they do in *I. baltica*.)
   - (*b*) Telson apical border not tridentate, almost invariably rounded laterally.
     - (i) Antenna shorter: segment 3 of peduncle, viewed from below, not longer than its maximum width: flagellum usually < ⅕ body length (Fig. 11). Antennule stouter (Fig. 1), the number of aesthetascs exceeding the length of the body in millimetres. ♂, adult appendix masculinus protrudes beyond 2nd pleopod (Fig. 7). Intertidal, amongst algae or barnacles.
       - (*aa*) Antenna very short: only segment 5 of peduncle longer than wide: flagellum markedly shorter than peduncle (Fig. 2). Telson broadly rounded; in adults with a small median point which is obtuse (Fig. 8). Colour mostly purplish with white patterning. Frequents exposed barnacle-covered rocks, sheltering in algal tufts, dead barnacles, etc. **I. pelagica** Leach

(*bb*) Antenna less short: peduncle with segments 4 and 5 longer than wide, and flagellum about as long as peduncle (Fig. 2). Telson narrower, with shallow concavity along its sides, its apical margin typically with a distinct median process which is acute (but rounded at the tip (Fig. 8)). Colour mostly red, brown or green. Large specimens frequent fucoids in the mid-tidal zone; smaller specimens amongst *Cladophora* etc. **I. granulosa** Rathke

(ii) Antenna longer: segment 3 of peduncle viewed from below longer than maximum width, flagellum equal to or more than $\frac{1}{5}$ body length (Fig. 2). [If the flagellum is about equal to $\frac{1}{5}$ body length, but the sides of the telson are slightly concave, turn to (i, *bb*) above.] Antennule more slender (Fig. 1), the number of aesthetascs equal to or less than body length in millimetres. ♂, appendix masculinus does not project beyond the 2nd pleopod in the marine species.

(*aa*) Body more slender, width $= \frac{1}{4}$ length, or less. Head narrow, only $1\frac{1}{2}$ times as wide as its median length. Coxal plates 3 and 4 not reaching posterior margin of segment. Antennule tip with single aesthetasc, longer than the rest (Fig. 1). Adult ♂ appendix masculinus extending to or just beyond tip of 2nd pleopod (Fig. 7). Colour mostly uniformly green or brown. Confined to brackish water, sometimes in pools above H.W. **I. viridis** Slabber

(*bb*) Body more robust, width $> \frac{1}{4}$ length. Head wider, at least $1\frac{3}{4}$ times as wide as long. Coxal plates 3 and 4 reaching posterior border of segment. Antennule with paired aesthetascs at tip (Fig. 1). ♂, adult appendix masculinus not reaching tip of 2nd pleopod (Fig. 7). Marine, from L.W.S. downwards, amongst weed and sometimes under stones. **I. neglecta** Sars

(N.B. The telson of this species is broader than in *I. viridis* and *I. granulosa*, but more pointed at the apex than in *I. pelagica*.)

Specific Descriptions

Idotea pelagica (Fig. 11)

Body short and stout; 3–$3\frac{1}{2}$ times as long as broad. *Cephalon* rather more than $1\frac{1}{2}$ times as broad as long in the mid-dorsal line; anterior and posterior borders slightly concave. *Antennule* extending well beyond the second, but hardly beyond the third, segment of the antennal peduncle; first segment not greatly expanded in relation to the other segments, which are all robust. *Aesthetascs* in pairs, numbering up to 16 in males and up to 12 in females; aesthetascs greater in number than the body length in millimetres. *Antenna* very robust; flagellum by no means as long as peduncle and less than $\frac{1}{6}$ body length; most segments of flagellum broader than long, and with pads of fine hairs in adult males; segments numbering up to about 9 in both sexes, with terminal joint very stout and about $\frac{1}{4}$ the length of the subterminal segment. *Coxal plates* fairly broad, widening posteriorly and reaching posterior border of segments 4–7. *Legs* all very robust, the terminal claw being relatively larger than that of any other species; all legs after the first have pads of fine setae on their inner surfaces, in adult males. *Abdomen* with broadly rounded *telson* having a short median, obtuse tooth; not keeled. *Appendix masculinus* extending beyond the tip of the 2nd pleopod for nearly $\frac{1}{2}$ its own length in small males (4–5 mm) and for $\frac{1}{4}$ its length in large males (10–11 mm).

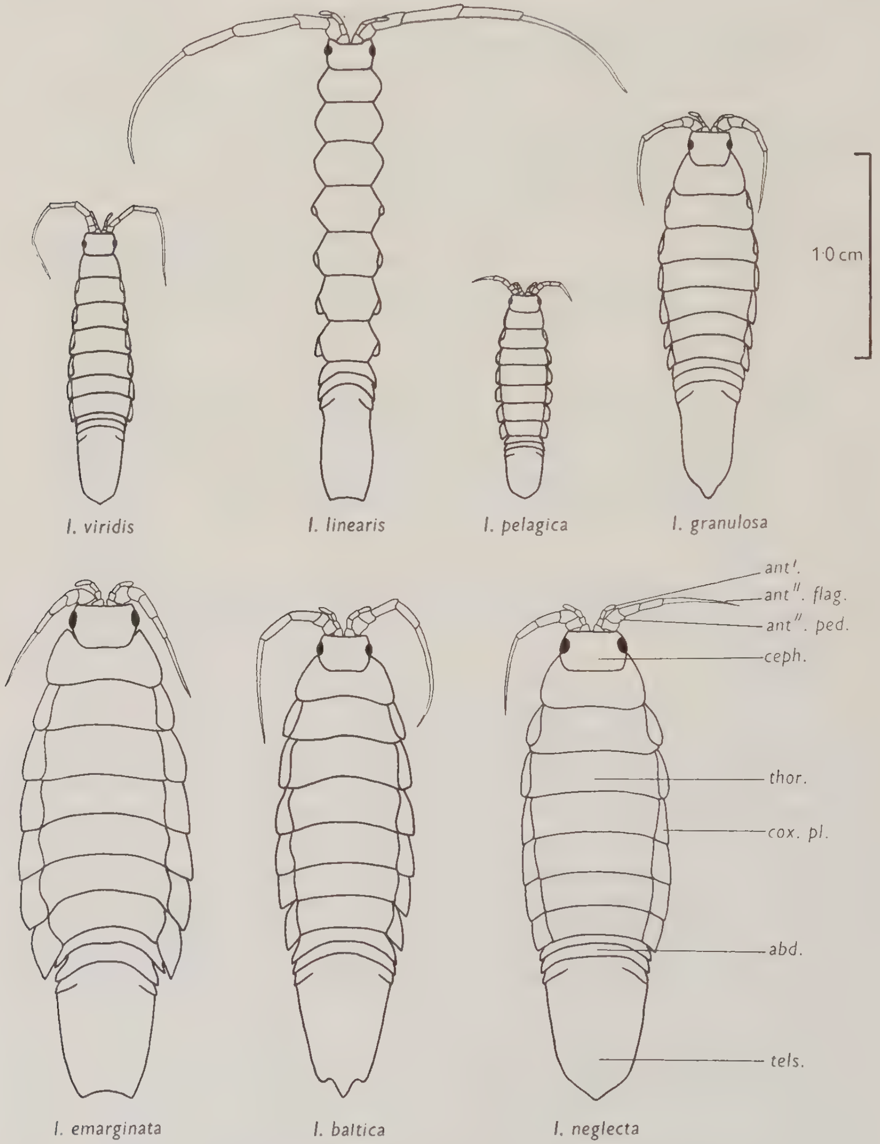
1·0 cm
I. viridis
I. linearis
I. pelagica
I. granulosa
ant'.
ant''. flag.
ant''. ped.
ceph.
thor.
cox. pl.
abd.
tels.
I. emarginata
I. baltica
I. neglecta

Fig. 11. Adult males of seven species of *Idotea*, seen from above.

Length at release from brood pouch 1–2 mm; males recognizable at about 4 mm, ranging to 11 mm; females 7–10 mm.

Colour: mostly dark purple-brown, with white diamond-shaped patches or elongated stripes down the mid-dorsal line, and with white markings along the edges of the dorsal side; females often darker than males.

Habitat: typically on shores exposed to wave action, where weeds are stunted and barnacles abundant. The colouring merges well with the background of barnacles.

Idotea granulosa (Fig. 11)

Body oval, narrowing rather sharply posteriorly; length often rather more than $3\frac{1}{2}$ times the width. *Cephalon* about $1\frac{1}{2}$ times as broad as long; anterior border almost straight, posterior border somewhat concave. *Antennule* extending beyond the second, but not beyond the third, segment of the antennal peduncle; first segment somewhat expanded, the second rather less so, and the others robust. *Aesthetascs* arranged in pairs, numbering up to 22 or more in males, and up to 16 or so in females; aesthetascs greater in number than the body length in millimetres. *Antenna* robust, flagellum not reaching length of peduncle (except possibly in largest males), and usually less than $\frac{1}{5}$ body length; segments of the flagellum numbering up to 16 in males, and 13 in females; largest males have tufts of setae on the flagellum; terminal segment pointed, and about $\frac{1}{3}$ the length of the subterminal one. *Coxal plates* fairly narrow, broadening posteriorly, and not extending to the posterior border until segment 4 and posterior ones. *Legs* robust; the second leg of adult males having pads of fine setae on the inner surfaces of the 4 most distal joints. *Abdomen* narrowing sharply at first, with rather concave lateral sides; *telson* with long, acute, median spine and very obtusely rounded lateral shoulders; not keeled. *Appendix masculinus* in smaller males projecting by about $\frac{2}{5}$ its own length, but only for about $\frac{1}{10}$ its own length in larger specimens (15–20 mm).

Length at release from the brood pouch 2 mm; some males recognizable at 5 mm, ranging to 20 mm or a little more; adult females 6–13 mm.

Colour: mostly uniformly brown, red or green, depending on the nature of the weed inhabited; occasionally with longitudinal white markings.

Habitat: predominantly a shore form, particularly abundant on *Ascophyllum*, and fucoids; smaller individuals prefer small weeds such as *Cladophora* and *Polysiphonia*. Occasionally on drift weed.

Idotea viridis (Fig. 11)

Body slender; length 4–5 times the width, though sometimes less, particularly in adult females. *Cephalon* about $1\frac{1}{2}$ times as broad as long; hardly concave, either anteriorly or posteriorly. *Antennule* extending well beyond the third peduncular joint of the antenna; first joint expanded, others slender.

Aesthetascs usually arranged in pairs except for a single one, slightly larger than the rest, situated at the extreme tip of the antennule (any other single ones are added to at the next moult); aesthetascs number up to 11 in males, and up to 9 in females. *Antenna* long and slender; flagellum longer than the peduncle except in very small forms, and about ¼ the body length; segments of the flagellum numbering up to 18 in males, and up to 13 in females; terminal style roughly cylindrical, about ½ the length of the subterminal segment. *Coxal plates* narrow, increasing in size posteriorly, and only those of segments 5, 6 and 7 reaching the posterior border of the segment. *Legs* long and very slender, second limb of adult males bearing pads of fine hairs. *Abdomen* with sides subparallel, slightly keeled posteriorly in the mid-dorsal line; *telson* with a single, median tooth, hardly acute, and with obtuse lateral corners. *Appendix masculinus* of most males projecting for about $\frac{1}{10}$ of its own length beyond the second pleopod, though hardly projecting at all in the largest males.

Length when first released from the brood pouch 1–2 mm; males recognizable often from about 5 mm, ranging to 15 mm; adult females range from 6 to 10 mm.

Colour: mostly uniformly green or brown, sometimes with white markings; females often darker than males, as with all species.

Habitat: prefers brackish water; often in brackish pools above H.W.N., which are not continuously reached by the sea. It is also recorded from the tidal region in estuarine conditions.

Idotea linearis (Fig. 11)

Body very slender; length 4–7 times the width, but mostly between 5–6 times. *Cephalon* nearly twice as broad as long; deeply concave anteriorly, and slightly so posteriorly. *Antennule* extending just beyond the second peduncular joint of the antenna; first joint very much expanded and rounded, others slender. *Aesthetascs* long and slender, arranged in pairs except for a terminal one separate from the rest; numbering up to 19 or more in adult males and 17 or more in adult females. *Antenna* very long and slender, peduncle rather longer than the flagellum; flagellum about ⅓ body length, having up to about 20 segments in males and rather less in females; terminal style pointed, less than ½ the length of the subterminal segment. *Coxal plates* very small, never extending to the posterior border of any segment. *Legs* long and slender, becoming somewhat palmate distally: in addition to the usual armature of spines, the second and succeeding legs of adult males have pads of fine setae on their inner borders. *Abdomen* with sides slightly incurved: *telson* with concave posterior border, and with a small median spine in young specimens. *Appendix masculinus* extending well beyond the tip of the second pleopod, by about ⅓ its own length, or rather less, in adult males.

Length at release from the brood pouch between 2 and 3 mm; males often recognizable from about 15 mm, ranging to over 20 mm in length; females rather less in size.

Colour: green or brown, with darker or lighter longitudinal stripes; adult female often darker than male, frequently with paler markings around the edges.

Habitat: usually sublittoral, associated with drift weed or bottom living algae, though occasionally found intertidally.

Idotea baltica (Fig. 11)

Body oblong oval; length usually more than 3 times the width. *Cephalon* width rather more than 1½ times length; somewhat concave anteriorly and posteriorly. *Antennule* extending to or just beyond the distal end of the 3rd segment of the antennal peduncle; 1st and 2nd joints expanded, others elongated and fairly robust. *Aesthetascs* in pairs, numbering up to about 20 in males and about 16 in females. *Antenna* slender, with flagellum longer than peduncle and about ¼ the body length; flagellum segments numbering up to 20 in males and 15 or so in females; terminal style pointed, and about ⅓ the length of the subterminal segment. *Coxal plates* large in adults, extending from anterior to posterior borders of all segments from the 2nd or 3rd backwards, becoming wider posteriorly. *Legs* fairly robust; 2nd leg of adult males having pads of fine setae. *Abdomen* dorsally keeled, with more or less straight sides, tapering to a tridentate *telson*; median process long and acute, lateral processes smaller (obtuse and less pronounced in young specimens). *Appendix masculinus* reaching just about the end of the 2nd pleopod in smaller males, but failing to do so by about $\frac{1}{10}$ of its own length in larger males.

Length about 2 mm in newly released juveniles; males first recognizable at about 10 mm, ranging to 30 mm; adult females 10–18 mm.

Colour: sometimes uniformly green or brown, but often with white marbling in the form of spots, or pale longitudinal lines, particularly along the edges; female often darker than male.

Habitat: generally sublittoral, but not infrequently found on the shore, e.g. on *Halidrys* at L.W.M.; often on drift weed.

Idotea neglecta (Fig. 11)

Body oblong oval; length a little more than 3 times the width. *Cephalon* rather more than 1½ times as broad as long: concave anteriorly and posteriorly. *Antennule* extending to (or nearly to) the distal end of the 3rd joint of the antennal peduncle; 1st and 2nd joints expanded, others elongated and fairly robust. *Aesthetascs* in pairs, numbering up to 12–14 in males, and 10–12 in females. *Antenna* fairly slender, flagellum longer than peduncle and ⅕–¼ the body length; flagellum segments numbering up to 20 in males and up to about 14 in females; terminal style pointed and averaging $\frac{1}{3}$ the length of the subterminal segment. *Coxal plates* wide, extending the whole length of the segment from the 2nd or 3rd to the last. *Legs* robust, the 2nd legs of adult males having pads of fine setae. *Abdomen* with sides fairly straight and converging posteriorly, with hind border of *telson* characterized by a median obtuse tooth, and obtusely rounded lateral shoulders; keeled. *Appendix masculinus* fails to reach the tip of the second pleopod by up to $\frac{1}{3}$ of its own length.

Length on release from the brood pouch about 2 mm; males recognizable from about 8 mm, ranging to nearly 30 mm; adult females range from 10 to 16 mm.

Colour: often uniformly brownish, sometimes with white longitudinal lateral markings, and occasionally with white marbling over the whole dorsal surface; adult females mostly darker than males.

Habitat: generally sublittoral, often on accumulations of organic matter rather than on attached weed; occasionally on surface drift weed.

Idotea emarginata (Fig. 11)

Body oblong oval; length hardly more than 3 times the width. *Cephalon* about $1\frac{3}{4}$ times as broad as long; both anterior and posterior borders concave. *Antennule* extending beyond the 3rd joint of the antennal peduncle; first and second joints expanded, others fairly robust. *Aesthetascs* in pairs; numbering up to 16 in males, and up to about 14 in females. *Antenna* somewhat robust, flagellum longer than peduncle and about $\frac{1}{6}$–$\frac{1}{5}$ the length of the body; flagellum segments numbering up to about 18 in males and 14 in females; terminal style pointed, and about $\frac{1}{3}$–$\frac{1}{2}$ the length of the subterminal segment. *Coxal plates* broad, extending over the whole length of the segment in segments 2 or 3 and posterior ones; becoming wider posteriorly. *Legs* robust; second leg of adult males having setose pads. *Abdomen* with sides curving slightly outwards, and with an emarginate *telson*, the lateral shoulders forming two obtuse points which are recognizable from a size of about 5 mm onwards; telson at first bluntly rounded, then cut straight across, then showing the emarginate character as growth proceeds. *Appendix masculinus* almost reaching the tip of the second pleopod in small males, though it fails to do so by up to $\frac{1}{4}$ of its own length in adult males.

Length when first released from the brood pouch about 2 mm; males first recognizable from 7–9 mm, depending on the season of release, and ranging to about 30 mm; adult females from 9 to 18 mm.

Colour: males, particularly, often uniformly brown in colour, though sometimes with white markings; females generally darker in colour, often with longitudinal lateral white bands, or alternating white and darker transverse bands.

Habitat: generally sublittoral on accumulations of algae, though occasionally on drift weed.

SUMMARY

Detailed descriptions of the external morphology, from the functional and developmental aspects, are given for seven species of *Idotea*. The taxonomic value of various structures is discussed in relation to previous work on the genus, and a key to the identification of *Idotea* is given. Specific descriptions are revised.

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ON THE BIOLOGY OF *CALANUS FINMARCHICUS*

VIII. FOOD UPTAKE, ASSIMILATION AND EXCRETION IN ADULT AND STAGE V *CALANUS*

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(Text-figs. 1–4)

INTRODUCTION

Apart from a few scattered observations (Hensen, 1887; Gran, 1902) the study of the food of *Calanus* began with Dakin (1908). He examined the remains present in the gut, and this was the method used also by Esterly (1916), Lebour (1922) and Marshall (1924). They all found that these remains consisted of a greenish mush containing the skeletons of numerous planktonic organisms, chiefly diatoms and dinoflagellates. Naked flagellates were, however, occasionally seen, and it was realized that the food might in reality consist largely of organisms which had no skeleton and could leave no recognizable remains.

The feeding of *Calanus* can also be studied in the laboratory by keeping the copepods in a suspension of food organisms and seeing if the organism is taken in, and if so whether or not the animal thrives on it. Such experiments have been made by numerous workers (Crawshay, 1915; Clarke & Gellis, 1935; Fuller & Clarke, 1936; Fuller, 1937; Harvey, 1937; and Clarke & Bonnet, 1939). Their criterion for assessing the value of an organism as food was usually either survival time or successful moulting. In female *Calanus* the food value of different organisms can also be tested by their effect on egg production (Marshall & Orr, 1952).

A simple method of finding out whether the food has been taken in or not is by observing whether faecal pellets are produced. This method was first used by Raymont & Gross (1942), and some data were also given by Harvey (1942).

In *Calanus* digestion takes place in the wide anterior part of the gut, and as the food mass passes into the narrow posterior part it is gradually compacted into a cylindrical pellet. When this is ejected, it is surrounded by a delicate pellicle which is sometimes produced into a 'tail' at one end. The pellicle may correspond to the chitinous one described by Forster (1953) on the faecal pellets of decapods.

Descriptions of the variation in size and shape of *Calanus* faecal pellets on several different foods are given by Raymont & Gross (1942) and Marshall & Orr (1955), together with some information on the numbers produced and the differences between male, female and Stage V. The size and shape of faecal pellets show great individual variation; indeed in an experiment in which two *Calanus* had been introduced by mistake into one dish, the two sets of pellets were easily distinguishable. The shape and size also depend on the food from which they are produced. With *Dunaliella* they are short and dark with an average length of about 500 μ for females and Stage V, and about half this for males. In general those produced with flagellates are dark but with *Dicrateria* and *Hemiselmis* the pellets tend to be smaller and paler than those with *Dunaliella*. With *Chromulina* the pellets are very sticky and difficult to deal with. With *Chlorella* they tend to fluff up and disappear. With diatoms the pellets are long and pale in colour; for example, with *Skeletonema* they average in females 950 μ in length. When *Calanus* is fed on thecate dinoflagellates such as *Prorocentrum* and small *Peridinium* spp. the presence of unbroken shells in the pellets gives them a characteristic granular appearance.

The size of the pellets is unaffected by the concentration of the food unless this is low, e.g. with a concentration of *Dunaliella* of 8 cells per mm^3, they measured about 300 μ instead of the usual 500 μ (Fig. 1 and Table V). When food is very scarce indeed a few pellets are still produced but these are 'ghosts' being very small and almost transparent.

In one experiment (Table I) 13 *Calanus* were kept individually in a food culture and examined at frequent intervals. The different females had different rates of faecal pellet production but these remained fairly constant over the 24 h.

METHODS

Two methods were adopted to study the food uptake of *Calanus*. The first was to count the number of faecal pellets produced under standard conditions. The individual variations can be very great, and to avoid this source of error experiments were made with duplicate or triplicate samples of 20 *Calanus*, each in a bowl containing about 400 ml. of the culture to be tested. The number of faecal pellets in aliquot samples was counted after a period of about 16–24 h. The volume per *Calanus*, 20 ml., was later found to be too low for optimum results (see p. 502).

Since the amount of food taken varies with the developmental stage and the season of the year, *Dunaliella* was used as the standard food and other cultures compared with it. When different species were compared as food an attempt was made to provide equal volumes of cell substance in the cultures used. This method, however, was superseded by the second, and results obtained by its means are used only to confirm or supplement later observations.

The second method was to use radioactive phosphorus (^{32}P) as a tracer element. Cultures of many phytoplankton organisms can be grown using ^{32}P as part source of the phosphorus necessary and these can in turn be fed to *Calanus*. The estimation of ^{32}P is rapid and extremely sensitive, and by this means the uptake, assimilation and excretion of *Calanus* can be accurately measured. Carrier-free orthophosphate in dilute hydrochloric acid was used as supplied by the A.E.R.E., Harwell, and from 0·25–2·0 mc was added to each litre of culture solution. When growth was good this gave a reasonably high activity by the time the cultures had reached a stage suitable for use. Many of the cultures were grown using only dilute modified Miquel solution (Ketchum & Redfield, 1938), but to others soil-extract or other growth-promoting substances were added. The cultures were considered suitable for experiment when most of the ^{32}P had been taken up by the cells. Phosphorus is an important limiting element for plant growth in the sea, and is also important in animal nutrition. It is present in all the major food constituents and should therefore give a good idea of the assimilation but some caution is necessary in interpreting the results.

It has been shown by various workers (Kamen & Spiegelman, 1948; Gest & Kamen, 1948; Goldberg, Walker & Whisenand, 1951; Rice, 1953) that when algae are grown in phosphate-rich media, some of the phosphorus in the culture organisms may be labile or very loosely bound. Rice has shown that in cultures with low P concentrations, only about 2% is exchangeable after they are a week old. In our cultures phosphorus was a limiting factor for growth, and as a rule they were not used until the ^{32}P in solution had dropped below 10% and sometimes below 5% of the total. On a few occasions when there was a considerable proportion of the ^{32}P in the filtrate, the organisms were filtered off or centrifuged, washed and re-suspended in membrane-filtered natural sea water.

Since it has been stated that the loosely bound phosphorus can be removed by washing, an experiment was made to find whether the percentage assimilated of the phosphorus in the culture was significantly lower after repeated washing. A culture of *Lauderia* was centrifuged (10 min at 2500 rev/min), the supernatant liquid siphoned off and the cells re-suspended in filtered sea water. This was done four times and the resulting suspension compared as food with the original, uncentrifuged culture, using the method described below. Since two-thirds of the cells had been lost, the original culture was diluted to give approximately the same cell content. The results are shown in Table II. In the centrifuged culture the percentage utilization varied from 87·2 to 93·5, in the untreated from 86·9 to 91·3, an insignificant difference.

After a considerable amount of trial and error, the following method was adopted.

Calanus, usually adult females, were picked out from a tow-netting and kept starved for several days in a cool room in the dark. It was found that they fed better and behaved more uniformly under such conditions than if they were taken from the sea and used for an experiment the same day. The number of cells in the radioactive culture was estimated, and dilutions made by adding sea water which had been filtered through a 'Gradocol' membrane of average pore diameter $0{\cdot}9\,\mu$. The usual dilutions were in the ratio 100:10:1. Five bottles of about 70 ml. capacity were used for each dilution and a single *Calanus* introduced into each. In addition, two bottles were filled with filtrate from the strongest by filtering it through a double layer of Whatman no. 42 paper. This was usually sufficiently fine to remove all the organisms. A *Calanus* was also added to each of the bottles with filtrate. When *Calanus* are kept in a solution containing inorganic ^{32}P, some of this is taken up in the body. The results from the two control *Calanus* gave a measure of this, enabling a correction to be made if necessary.

Each bottle was then tied in a dark cloth bag and attached to a wheel which was rotated slowly in a vertical plane (about one revolution in 2–3 min) to avoid settling out of the food organisms. The slow rotation is not likely to have any effect on the behaviour of the *Calanus*. Samples of the culture dilutions and of the filtrate were taken in triplicate or quadruplicate with a 0·2 ml. delivery pipette and dried on a metal disc (planchette) for measurement of the activity.

After a period, usually of about 16–24 h, the experiment was stopped. The *Calanus* was removed from each bottle, washed in three changes of sea water, transferred to a disc in a small drop of water, roughly torn up to avoid self-absorption of the ^{32}P and dried slowly on a warm plate. The radioactivity was then measured. On many occasions a sample of the final wash water was also tested.

The contents of each bottle in turn were then transferred to a rectangular Perspex dish with the inside angles bevelled to facilitate examination of the whole of the bottom. The dish was placed on a Perspex sheet marked off in squares and the faecal pellets and eggs (if present) removed under a binocular microscope. These also were washed three times, transferred to discs and dried for measurement of the activity. A sample of the final wash water was tested in each case.

In some experiments many of the faecal pellets were found broken or crushed at one end. It seems as if the *Calanus* must encounter a considerable number of them when swimming and must damage them. Any appreciable loss of ^{32}P after the pellicle is broken might cause a serious error in the results. To find if this was important nine sets of faecal pellets (20–60 in each) some whole and some broken, were left overnight on coverslips in a drop of water. In the morning the water was withdrawn and tested as well as the faecal pellets. The radioactivity of the water was found to be only just over 1 % of that of the pellets and the loss is therefore negligible. When faecal pellets are broken, small fragments may be lost during washing, and although this loss was never serious, it will tend to make digestion results appear higher.

A description of the method of measuring the radioactivity is given in this *Journal* by Spooner (1949). The discs were exposed in a lead castle at a fixed distance from the aluminium end-window of a Geiger-Müller counter and the impulses counted on a scaling unit. Corrections were made for quenching time, distance from the end-window, background and, when the experiment lasted for more than a day, for decay as well. The results can be expressed either as number of pulses per minute, or, since the activity of the culture and the number of cells are known, as cell equivalents.

In Table III are shown the results of a typical experiment with *Skeletonema costatum* culture. It shows a number of characteristic features. In the first place there is a considerable variation in the behaviour of the individual *Calanus*. Some fed well, some not so well and one not at all. Secondly, the volume filtered was much lower at the highest concentration. The number of faecal pellets was approximately the same at the two higher concentrations and considerably lower in the lowest. The cell equivalents per faecal pellet were approximately the same throughout, although this was not often the case in other experiments. The percentage of the phosphorus-containing portion of the diatom utilized showed no significant difference at the different dilutions.

The rate of filtration, F, of the *Calanus* can be calculated (Gauld, 1951) according to the formula

$$F = v\,\frac{\log_{10} C_0 - \log_{10} C_t}{t \log_{10} e},$$

where v is the volume of the bottle in ml., t the duration of the experiment in days, C_0 the original and C_t the final concentration of the food organisms. The initial concentration C_0 was obtained by measuring the activity in aliquot samples of the culture used and the final concentration C_t was obtained by subtracting from this figure the activity in the body, eggs and faecal pellets of the *Calanus*.

It should theoretically be possible to obtain the same results by measuring the activity in an aliquot sample from the bottle at the end of the experiment or by counting the cells in the bottle at the beginning and end of the experiment. The last method is less accurate because the error of counting cells is relatively high and the count has to be multiplied by a large figure owing to the smallness of the sample. Similarly, by counting the pulses in the culture at the end by the usual method an error within the normal variation of the count may sometimes cause a large difference in the calculated volume filtered. Since the substance of the cells removed from the culture is concentrated in the body, eggs and faecal pellets of the *Calanus*, this figure is not subject to the same error.

The results of an experiment with *Skeletonema costatum*, in which all three methods were used, is shown in Table IV. The cells were pale and the counts were not very satisfactory; the ten initial counts (each of 3·2 mm^3) varied from

24 to 53, although the final counts were more uniform. Using the final cell counts of the three samples in which filtration was highest, the figures are of the same order of size as by the normal method. For counting the activity of the culture at the end a Veall liquid counter (1948) was used with 2 ml. diluted to 10 ml. in each case. The three sets of figures (ml. filtered in 24 h) show a rough agreement but that based on the activity of the *Calanus* body and faecal pellets is likely to be the most accurate.

In other balance experiments, using *Skeletonema costatum*, *Chaetoceros decipiens* and *Syracosphaera elongata*, the agreement was even less satisfactory.

EFFECT OF DIFFERENT FACTORS ON FOOD UPTAKE

Concentration of food

If *Calanus* is purely a filter feeder the number of faecal pellets produced should rise with increasing concentration of food. This has been noted in the sea for copepod pellets in general (Harvey, Cooper, Lebour & Russell, 1935).

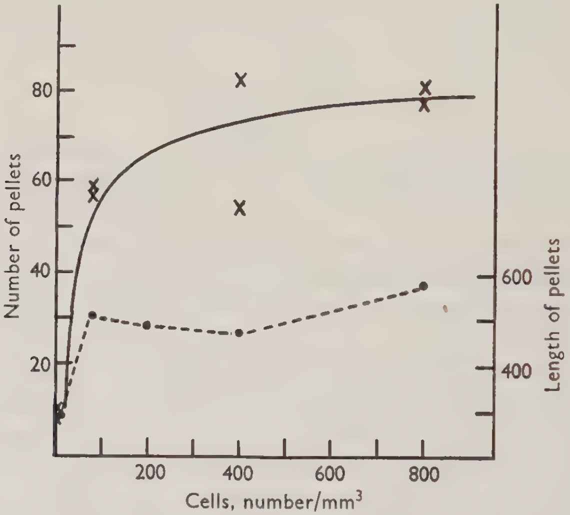

Fig. 1. Effect of concentration of *Dunaliella* on faecal pellet production. ——, number of pellets produced in 24 h; - - -, length of faecal pellet in μ.

Many experiments were made by keeping *Calanus* in bowls containing varying concentrations of the same culture, the number of faecal pellets produced being counted at the end of the experiment. The number usually did increase with increasing concentration but only up to a certain point varying with the organism used. Above this point the number did not increase regularly. The result of one experiment with *Dunaliella* is shown in Fig. 1 and Table V.

It appears that if *Calanus* is a filter feeder, it is not an automatic one but is able to slow down or stop feeding in high concentrations. This confirms observations made by Gauld (1953) that *Calanus* can remain for a long time in a thick suspension of cells, even swimming normally, without feeding.

In most of these experiments there was a tendency for the organisms to settle out or, in the case of some flagellates, to aggregate. The *Calanus* too, especially the females, have a habit of sinking to the bottom of the vessel and rummaging about there, and in dishes where there has been settling out they will get more than the calculated amount. This source of error was avoided in the later experiments with labelled phosphorus by putting the *Calanus* in closed bottles on a rotating wheel.

In a poor concentration of food where few cells are available they will often ingest threads from filter-paper or other such objects and the number of faecal pellets will be higher than one would expect.

When different concentrations were used in an experiment with labelled cultures the highest was much in excess of what is ever found in the sea; the next strength, 10% of this, was equal to or greater than what would be a rich concentration in the sea. Finally it was hoped that the third strength (usually 1%) would represent a moderate concentration in the sea.

In the highest concentration the volume filtered was often lowest. The number of faecal pellets produced in the 10% was always more than a tenth of that in the 100% and was often nearly as high. In the 1% the number produced was usually more in proportion to the concentration of cells, but it was often very low and with a very small number of faecal pellets the loss of one can make a considerable difference to the results. Figures for the weakest concentration may therefore be less accurate; the percentage digested will appear higher and the volume filtered lower than they should be.

To study the feeding of *Calanus* in very low concentrations of cells, such as are found in the sea in winter, a culture of *Chaetoceros decipiens* was grown with a quantity of ^{32}P much larger than usual (1 mc in 200 ml.). With this culture much greater dilutions could be used and reliable counts of pulses still obtained. Even in concentrations as low as 1 cell per ml. and in bottles of 150 ml. capacity the volume filtered in 24 h remained low (under 10 ml.). Similar results were obtained with a much larger diatom, *Ditylum brightwellii* (Table XIV).

Temperature

Most of the experiments were done at room temperature which varied from 10 to 20° C and was always somewhat above sea temperature at the same time. By counting the number of faecal pellets produced by *Calanus* kept at temperatures varying from 5 to 15° C it was found that there was usually a rapid rise in numbers between 5° and 10° C and a lesser rise between 10° and 15° C but the results were variable.

Volume of Container

It was found that the volume of the vessel in which the *Calanus* were kept had an effect on their feeding. In one experiment 15 females were kept individually in dishes of three different volumes (15, 50 and 100 ml.) containing the same culture of *Skeletonema*. These were not stirred during the 16½ h of the experiment. The *Calanus* showed considerable individual variation, but the volumes filtered in the 15 ml. dishes were decidedly lower (average 1·3 ml./24 h) than in the 50 or 100 ml. dishes (average 7·8 and 7·9 ml./24 h). Later experiments were therefore done in bottles of 70 ml. capacity or, in a few cases, 35–40 ml.

Light

It is well known that sunshine or even diffuse light out of doors is lethal to *Calanus* (Huntsman, 1924; Klugh, 1929, 1930); it also affects the respiration (Marshall, Nicholls & Orr, 1935). The harmful effect of bright light reduced the uptake of food in *Calanus* exposed to it (as judged by faecal pellet production), but those of the first generation of the year, which in the Clyde sea area live at the surface, are more resistant to its effects than those of the overwintering generation.

Experiments with radioactive cultures of *Syracosphaera* and of *Lauderia* showed that even in diffuse light indoors the volume of culture filtered was less than with *Calanus* kept completely in the dark. The results with *Syracosphaera* are shown in Table VI. These experiments were done in February in a well-lit room with windows on both sides and overhead, whereas before this all the radioactive feeding experiments had been done well away from the window in a north-facing room. The rather dim diffuse light there is not likely to have affected the *Calanus* appreciably, but subsequently all the experimental bottles were tied in black cloth bags. Since *Calanus* feeds more in the dark than in the light it appears that they do not catch their food organisms by sight.

Age of Culture

It is known that the chemical composition of the cells in a culture may vary with age and with the nutrients present. It is only during the exponential stage of growth that the composition remains approximately constant (Fogg, 1953). Our cultures were almost all grown in sea water enriched with a modified Miquel solution, but it was not possible always to use them at the same age and the amount digested may vary with the growth of the culture.

A number of experiments were made with a single culture of the diatom *Lauderia borealis* at different stages of its growth, and Table VII shows that the percentage utilized decreased with age. The dilutions used in the experiment varied but this did not affect the utilization appreciably. Finally, the old culture was compared with a new young culture of approximately the same cell concentration and the utilization of the latter was found to be a good deal higher.

In a young diatom culture during the phase of rapid multiplication the cells remain suspended, and it is during this period that the utilization by *Calanus* is high. With the *Lauderia* used this period was about 5 days. The cells then sank to the bottom and became smaller, paler in colour and were united in shorter chains than in a young culture. The change in the amount assimilated indicates that there is a corresponding change in the phosphorus-containing substances built up by the cell.

A later series of experiments with a culture of *Chaetoceros decipiens* up to 18 days old showed only a very slight effect. Similar experiments on a culture of a coccolithophore *Syracosphaera elongata* 16 and 33 days old showed no fall in digestibility, although by the thirty-third day the cells were depositing coccoliths in quantities.

Size of Food Cells

In discussions on the question of the food organisms available and suitable for *Calanus* in the sea much stress has been laid in recent years on the minute flagellates which form an important food of pelagic organisms such as larval molluscs. It was found by Ussing (1938) that the minimum distance between the finest setules on the maxillule in female *Calanus* was 5·7 μ. He suggested that the smallest organisms *Calanus* could filter off must have their greatest diameter more than this. Raymont & Gross (1942), however, kept *Calanus* alive and healthy on a diet of minute flagellates. The possible importance of bacteria also was suggested by Clarke & Gellis (1935), though later Fuller & Clarke (1936) concluded that bacteria were not important as food. The concentrations used were higher than would be expected in the sea.

Various organisms of different sizes were used in feeding experiments on *Calanus* (see Tables XIV–XVI), and the volumes filtered in 24 h are shown in Fig. 2. It is apparent that for organisms below a size of about 10 μ there is a very much lower filtration rate than for organisms of greater size. In the experiments with very small flagellates it was noted that the volume filtered was often greatest in the highest concentration instead of in the lowest as is more usual. This is what might be expected if the cells in a very high concentration gradually clogged up the filtering setae. In calculations of the volume filtered a complete filtration of the food organism is assumed and if it can to any extent pass the filter the calculation is unreliable.

A possible explanation of the failure to take small flagellates might be that they were all unpalatable to *Calanus*, and a search was made for some inert particle of uniform size which could be used instead. Indian ink and carmine are both ingested to a certain extent but the size of the particles is very variable. Dr D. W. Henderson of the Experimental Station, Porton, kindly supplied us with a suspension of radioactive spores of *Bacillus globigii*, 0·7 μ^3 in volume, and Table VIII shows the results of an experiment made with them. Before use the suspension was washed twice by centrifuging and then resuspending in membrane-filtered sea water. It will be seen that the volume filtered is extremely low. The small amount apparently digested is probably due to the spores and faecal pellets still in the gut of the *Calanus* at the time it was killed. It is clear that *Calanus* is unable to filter such a small particle efficiently.

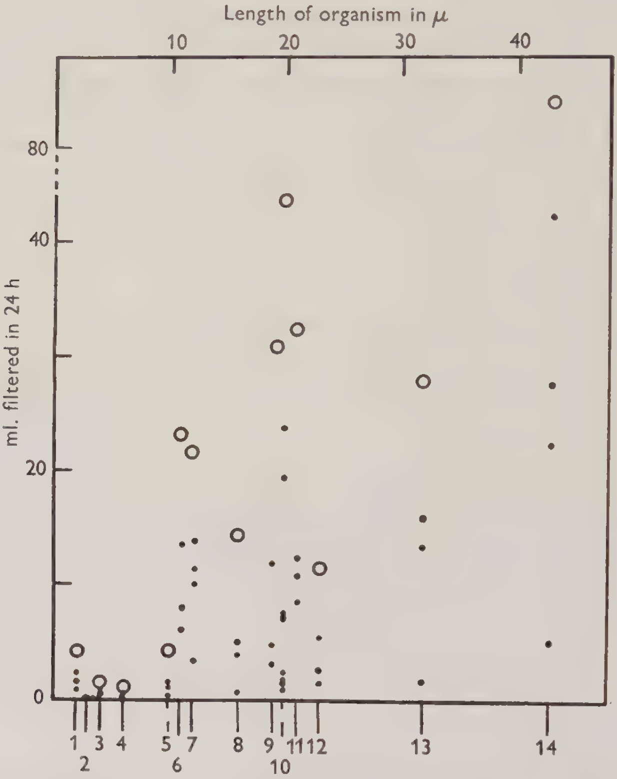

Fig. 2. Relation of size of food cell to volume filtered. ●, average with each concentration used in feeding experiments; ○, maximum volume filtered by a single *Calanus*. (1) *Chromulina pusilla*, (2) *Nannochloris oculata*, (3) *Dicrateria inornata*, (4) *Monochrysis lutheri*, (5) *Prymnesium parvum*, (6) *Gymnodinium vitiligo*, (7) *Prorocentrum triestinum*, (8) *Gymnodinium veneficum*, (9) *Chlamydomonas pulsatilla*, (10) *Syracosphaera elongata*, (11) *Cryptomonas* sp., (12) *Platymonas carteriaformis*, (13) *Oxyrrhis marina*, (14) *Prorocentrum micans*.

Dr M. R. Droop suggested that although the small flagellates might not be taken directly, they might be eaten by larger flagellates which in turn can be eaten by *Calanus*. A culture of the small alga *Nannochloris* (2–4 μ in diameter) which was itself very little used by *Calanus* was inoculated with a larger flagellate *Oxyrrhis*, which rapidly increased in numbers by feeding on the *Nannochloris* and was then used in a feeding experiment on *Calanus* (Table XVI). The volume filtered and the percentage assimilated showed that the *Nannochloris* could thus be utilized indirectly.

Harvey suggested in 1937 that in a mixture of large and small cells *Calanus* would select the large, and some experiments were made with labelled cultures to test this. The *Calanus* were given a mixture of a very small flagellate (*Dicrateria inornata* 3–5·5 μ) and a larger organism. Previous experiments had shown that in a unialgal culture of *Dicrateria*, *Calanus* produced only a few faecal pellets and filtered only a small volume of water. Feeding was compared in Dicrateria alone, in a mixture of *Dicrateria* of about the same concentration with a large diatom *Lauderia*, and in *Lauderia* alone. Since only the *Dicrateria* was radioactive, feeding in *Lauderia* could be measured only by the number of faecal pellets produced.

The results are shown in Table IX. When *Lauderia* was present nearly five times as many faecal pellets were produced as with *Dicrateria* alone, the amount of *Dicrateria* assimilated was nearly four times as much and over five times the volume of water was filtered. This could be explained by supposing that the *Calanus* in its preference for *Lauderia* was unable to reject the *Dicrateria* which was with it. An alternative explanation is that *Dicrateria* is too small to be efficiently filtered, but that the mucus exuded in cultures of diatoms entangles the cells so that they are ingested with the diatoms. That the second is the more probable explanation was shown by an experiment using *Prorocentrum micans* instead of *Lauderia*. This dinoflagellate exudes much less mucus than *Lauderia* and although the quantities used were greater, the difference between the unialgal and the mixed culture was not marked. With these organisms then it seems that *Calanus* is unable to select the larger from a mixture.

Another experiment was made with *Ditylum brightwellii* and *Chaetoceros decipiens*, two of the genera used by Harvey. Parallel sets were used, one having radioactive *Ditylum* and non-radioactive *Chaetoceros*, the other having radioactive *Chaetoceros* and non-radioactive *Ditylum*. The *Ditylum* used was about fifty times the volume of the *Chaetoceros*. If *Calanus* had shown a preference for the larger of the two diatoms, the volume filtered in the set with radioactive *Ditylum* should have been much the greater, but as will be seen in Table X there is no marked difference between the two.

Choice of Food

To find out whether *Calanus* could acquire a preference for one species rather than another, irrespective of size, a series of experiments was made by keeping *Calanus* in two different kinds of food, and after a few days putting each set in a mixture of the two foods. Organisms were chosen whose skeletons could be easily recognized in the faecal pellets, and the first experiments were done by examining these faecal pellets.

When a *Calanus* is transferred from one kind of culture into another the change over in the skeletons in the faecal pellets may not be complete until the third pellet has been extruded.

In a qualitative experiment using *Calanus* which had been kept in cultures of *Coscinodiscus centralis* and *Prorocentrum micans* it was found that after some hours in a mixture of the two both lots had taken *Prorocentrum* freely, but that *Coscinodiscus* fragments were present in about two-thirds of the faecal pellets of those fed originally on *Coscinodiscus* and in only one-fifth of those fed on *Prorocentrum*. The diatoms sank to the bottom of the dish while the flagellates swam freely and this may have affected the results. After 3 days, however, there was no difference between the pellets from the two lots. These experiments suggest that there may be for the first hour or two some power of selection.

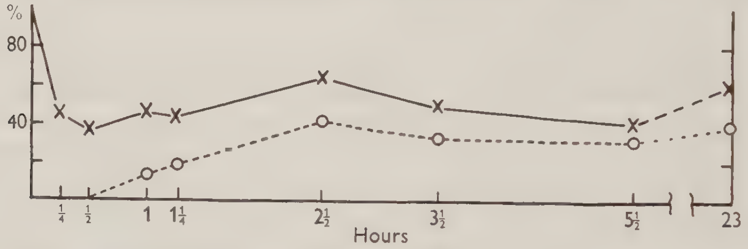

Fig. 3. Choice of food experiment. *Calanus* feeding in a mixture of *Peridinium trochoideum* and *Prorocentrum micans*. ——, % *Prorocentrum* in the faecal pellets of those accustomed to *Prorocentrum*; - - -, % *Prorocentrum* in the faecal pellets of those accustomed to *Peridinium*.

Two experiments were then carried out in a similar way comparing Stage V *Calanus* accustomed to *Prorocentrum micans* and *Peridinium trochoideum* respectively. The results were similar but only the second, in which improvements in method were made, will be described (Table XI and Fig. 3). The *Calanus* which had been kept for 10 days in their respective cultures were put singly into dishes containing about 15 ml. of a mixture with about 900 cells of *Prorocentrum micans* and 3900 cells of *Peridinium trochoideum* per ml. This mixture was chosen in an attempt to give approximately equal volumes of each food. The dishes were examined at frequent intervals and the faecal pellets produced by each *Calanus* removed (Table I). These were squashed individually under a coverslip and the skeletons of both organisms counted. The skeletons as a rule remained whole and were easily counted, but occasionally they were so numerous or so many had split into halves that the number was not very accurate. When the number of faecal pellets was small all were examined but when it was large a sample of three was taken from each *Calanus* and the total number calculated. Individual variation in feeding rate and in the proportions eaten was marked and the average is therefore given. As Fig. 3 shows, there is a considerable difference in the behaviour of the two sets. Those accustomed to *Prorocentrum micans* ate equal numbers of the two species from the first half hour onwards whereas those accustomed to *Peridinium trochoideum* did not begin to eat *Prorocentrum micans* till after half an hour had elapsed and even then the proportion was low. After 2½ h they reached a fairly steady state. The numbers and not volumes are shown in Fig. 3 and Table XI. If we allow for volume there appears to be a preference for *P. micans*, much more marked in those accustomed to *P. micans*.

The number of organisms whose skeletons remain whole and can be counted easily in faecal pellets is very limited, but by growing cultures with ^{32}P one can use foods which cannot be thus recognized. A much more accurate measure of the cells taken up can also be made.

Feeding experiments were done by keeping *Calanus* in two different food cultures and then measuring their uptake in a mixture of the two, only one of which was radioactive. One such experiment compared the utilization of a large diatom (*Lauderia borealis*) with a small (*Skeletonema costatum*). From measurements of the cells in the culture it was calculated that a *Lauderia* had 122 times the volume of a *Skeletonema* cell. Six of each set of *Calanus* were kept in the mixture for ½ h, six for 1 h, six for 2 h, and six for 16 h. A summary of the results is shown in Table XII, and it is clear that there is no significant difference between the two sets.

A similar experiment was made with *Calanus* (fed for a week previously) using *Lauderia* and radioactive *Syracosphaera*, and again there was no significant difference between the two. In a third experiment using radioactive *Lauderia* and *Cryptomonas* (Table XIII) the *Calanus* which had been kept in *Lauderia* did for the first hour eat more *Lauderia* than those kept in *Cryptomonas*, although this difference was not observed in the 2 h samples.

The results of the experiments with ^{32}P do not confirm either Harvey's observations or the results we obtained by counting skeletons in faecal pellets. If selection takes place it does not do so to a marked degree. It is difficult to understand the mechanism of selection since, as has already been mentioned, *Calanus* feeds better in the dark than in the light.

Utilization of Different Species

A variety of different organisms was tested to find their value as food. They were chosen from the main groups found in the phytoplankton and included a variety of size from the 1–3 μ of *Chromulina* to the 100 μ or so of *Coscinodiscus*. Special attention was paid to the very small flagellates since it has often been suggested that they may be of outstanding importance as food for copepods.

Some of these are open sea forms and others were isolated from shore pools.

The following organisms were used:

CHLOROPHYCEAE

Dunaliella sp. (Plymouth strain 81)
Chlamydomonas pulsatilla Wollenweber
Platymonas carteriaformis nom. prov. (M. R. Droop's strain 10)
Chlorella stigmatophora Butcher (Plymouth strain 65)
Nannochloris oculata Droop (M. R. Droop's strain 66)

CHRYSOPHYCEAE

Chromulina pusilla Butcher (Plymouth strain 90)
Monochrysis lutheri Droop (M. R. Droop's strain 60)
Dicrateria inornata Parke (Plymouth strain B)
Pseudopedinella sp. (Plymouth strain 91)
Coccolithus huxleyi (Lohmann) Kamptner (Plymouth strain 92)
Hymenomonas carterae (Braarud & Fagerl.) Braarud (Plymouth strain 17)
Syracosphaera elongata Droop (M. R. Droop's strain 62)
Prymnesium parvum Carter (M. R. Droop's strain 65)

BACILLARIOPHYCEAE

Coscinodiscus centralis Ehrenberg (Plymouth strain 105)
Thalassiosira gravida Cleve (Plymouth strain 112)
Lauderia borealis Gran (Plymouth strain 111)
Skeletonema costatum (Grev.) Cleve (Plymouth strain 106)
Rhizosolenia delicatula Cleve
Chaetoceros decipiens Cleve (Plymouth strain 107)
Ditylum brightwellii (West) Grun. ex Van Heurck (Plymouth strain 110)
Nitzschia seriata Cleve (Plymouth strain 124)

CRYPTOPHYCEAE

Cryptomonas sp. (Plymouth strain 23)
Hemiselmis rufescens Parke (Plymouth strain D)

DINOPHYCEAE

Exuviaella sp. (Plymouth strain 18)
Prorocentrum micans Ehrenberg (Plymouth strain 97)
P. triestinum Schiller (Plymouth strain 98)
Oxyrrhis marina Dujardin (M. R. Droop's strain 18)
Gymnodinium vitiligo nom. prov. Ballantine (Plymouth strain 102)
G. veneficum nom. prov. Ballantine (Plymouth strain 103)
Peridinium trochoideum (Stein) Lemm. (Plymouth strain 104)

Diatoms

Table XIV shows all the experiments made by using cultures grown with ^{32}P. All the diatoms tested were eaten freely and the size of the cell had apparently no effect on the amount taken up. Since diatoms normally occur in chains even those with very small cells will be easily filtered.

With the four species in which assimilation was measured the proportion digested was high, the average being always over 50% and usually over 80%. The percentage did not vary appreciably with the concentration, the apparent slight rise in low concentrations having perhaps been caused by the incomplete recovery of faecal pellets. The percentage is lowest in the spring diatom *Skeletonema* and considerably higher in the three others. Yet *Skeletonema* is undoubtedly the most important diatom food in the Clyde sea area.

As is to be expected the actual quantity of food taken up is greatest when the concentration is highest and this is true also of the number of faecal pellets produced. The quantities are intermediate in the middle concentrations and least in the lowest concentrations, but, as has already been pointed out for faecal pellets, the relationship is not linear. One would therefore expect the volume of water filtered to be lowest in the high concentration and this is usually the case. It must be admitted, however, that in the experiments this volume shows a very great variation, not only among individual *Calanus* but between different experiments on the same species.

Flagellates

Table XV gives the results of the feeding experiments with cultures of flagellates grown with ^{32}P. The relation of volume filtered to size has already been discussed.

The percentage digested is high in most cases, but there are exceptions in *Dicrateria inornata*, *Chromulina pusilla* and possibly *Nannochloris* sp. in which so few faecal pellets were produced and so little taken up in the body that the results are unreliable.

The two very similar forms, *Dunaliella* sp. and *Chlamydomonas pulsatilla* were used. The second is considerably the larger and as can be seen in the table it is a good food. The first is a little smaller and has also been shown by experiments on egg production (Raymont & Gross, 1942; Marshall & Orr, 1952) to be a good food at least when present in high concentrations, but it has the disadvantage that it aggregates readily and is therefore difficult to sample accurately.

Although *Chlorella* can be taken in by *Calanus*, the faecal pellets produced are peculiar in that they seem to consist mainly of a mass of unaltered cells. That some of these at least are viable was proved by Dr M. R. Droop, who grew a culture of *Chlorella* from a faecal pellet. When the pellets are punctured or broken (and they seem to be particularly fragile) a cloud of cells emerges and the whole pellet soon disappears. *Chlorella* is therefore unsuitable for quantitative work since by the end of an experiment many faecal pellets may have completely disintegrated. In one or two experiments where the *Calanus* were kept in small crystallizing dishes and the faecal pellets removed at frequent intervals, the percentage digested was low, usually below 50% and often only 15 or 20%. The food present in the gut probably accounts for most of the small amount found in the body so that digestion is even lower than it appears to be.

Dinoflagellates

Table XVI shows the results of all experiments with radioactive cultures of dinoflagellates. That with *Peridinium trochoideum* was not done as usual in rotated bottles, but in 50 ml. crystallizing dishes and therefore the figure for volume filtered may not be so reliable.

On the whole dinoflagellates are, with the possible exception of *Prorocentrum triestinum*, digested well. The volume filtered is also high and as one might expect with fairly large cells rises with lowered concentration. Indeed the highest figure we have obtained for volume filtered (84 ml.) was got with one out of the five *Calanus* in a culture of *Prorocentrum micans* containing 5 cells per ml.

The culture of *Oxyrrhis* used (see p. 505) also contained a large number of very small *Nannochloris* cells on which they were feeding. Table XV shows, however, that even in much higher concentrations the amount of *Nannochloris* actually ingested is negligible, so that the results obtained can be considered as due to *Oxyrrhis* alone. This is shown also by the fact that there is an increase in volume filtered with increasing dilution.

Prymnesium parvum is interesting in that, under certain conditions of growth, it is poisonous to fish, both in the sea (Otterstrøm & Steemann Nielsen, 1939) and in fish ponds (Shilo & Aschner, 1953). With *Calanus*, however (Table XV) a culture grown by Dr M. R. Droop showed no evidence of being harmful. *Gymnodinium veneficum*, which has been found to be poisonous to fish by Miss D. Ballantine of the Plymouth laboratory (personal communication), was also tested on *Calanus* (Table XVI). In rich cultures it caused death, but only after one or more days, which is much slower than with fish. That the *Calanus* ate it was shown by the production of faecal pellets. Another culture grown with ^{32}P present was tried in a quantitative feeding experiment, using lower concentrations. Few of the *Calanus* fed freely but in those which did, faecal pellet production was normal and digestion high.

DIGESTION

When faecal pellets taken in tow-nettings are teased out and examined they sometimes appear to consist largely of unaltered cells of diatoms and other organisms. This, and the rapid production of faecal pellets in cultures in the laboratory, led observers to suppose that digestion went on only to a limited extent.

For the experimental feeding of *Calanus* it is possible to choose food organisms which give rise to faecal pellets in which the skeletons of the food organisms can be counted and the degree of digestion assessed visually. One such experiment was made by feeding *Calanus* on *Prorocentrum micans*. In the gut the *Prorocentrum* readily breaks up into two halves, and it is possible to see whether these are empty or if they contain an appreciable amount of undigested material. The concentrations ranged from 33 to 1·7 cells/ml. and the dilutions were made with outside sea water which contained at that time a variety of other organisms but no *P. micans*. In addition, the culture contained about 25 % of empty shells which were also presumably ingested by the *Calanus*. Five sets each of fifteen *Calanus* were used and the faecal pellets produced were counted. A sample of the faecal pellets from each was crushed, the *Prorocentrum* shells counted and the number fully digested assessed. This varied from 66 % in the highest concentration to 100 % in the lowest. Estimates of degree of digestion are of course not very reliable. Even when most of the shells were empty, there was still a mass of greenish material sometimes retaining the shape of the original cell body present in the faecal pellet and this was probably only partly digested.

By the use of cultures grown with ^{32}P it was possible to estimate the digestion of a large variety of organisms, and these results are shown in Tables XIV–XVI. Although in most cases digestion appears to be very high, there are exceptions. The percentage digested is usually between 50 and 100 % but very low results were obtained with *Dicrateria*, *Chromulina* and *Chlorella*, as well as with the spores of *Bacillus globigii* (p. 504).

From the single experiment on non-radioactive *Prorocentrum micans* it did appear that there was a tendency to digest more in low concentrations. This tendency is not confirmed in the experiments with radioactive cells, for the differences in the percentage digested between the richest and poorest concentration is usually within experimental error.

Faecal pellets are sometimes produced at the rate of one every 5 or 6 min, and it is astonishing that, in these conditions, digestion of ^{32}P is still as high as 80 or 90 %. A low content of phosphorus in copepod faecal pellets, as compared with the phytoplankton was also found by Harvey *et al.* (1935), although they thought that the phosphorus was liberated directly into the sea as phosphoproteins and phospholipins.

Nearly all the feeding experiments were made with starved females, and it seemed possible that when first fed after a period of starvation digestion might be more efficient. A number of females was therefore kept for 6 days in a rich culture of *Chaetoceros* before use. They produced a large number of faecal pellets and when used for a feeding experiment the percentage digested was still found to be over 90 % and the volume filtered was 24–46 ml. per *Calanus* in 24 h.

DISTRIBUTION OF ^{32}P IN THE *CALANUS* BODY

Rough dissections of numerous *Calanus* were made to determine the fate of the ^{32}P taken in. The *Calanus* was fixed in a drop of formalin and then dissected with needles under a binocular microscope on a series of circular coverslips of the same diameter as the planchettes used. These were then dried and their activity measured. The phosphorus begins to leach out immediately after fixation, and up to 12% may be lost overnight so the dissections were done within a few hours of fixation.

The fat was separated off as far as possible on the first coverslip, and then the *Calanus* was transferred to a second and most of the gut taken out and put on a third. The reproductive system was then dissected out. After this the carcass was transferred to a fourth coverslip and the muscles, apart from those inside the appendages, removed to a fifth. As can be well understood the separation of the different tissues was by no means complete. When fat was present (always in Stage V but rarely in females) it was never possible to leave it all on the first coverslip and the whole series contained some. The body fluid came out on the first (fat) or second (gonad) coverslip. It is difficult to disentangle the oviduct from the muscles to which they are attached and some eggs are left with the carcass. Otherwise this consists of the exoskeleton, appendages with their contained muscles, and the nervous system. On several occasions most of the nerve cord was removed and read separately, but the ^{32}P content was very small.

In spite of the crudity of the method, the results were fairly consistent (Table XVII). In females without fat, the carcass and the reproductive system each contained roughly 30–50% of the whole, the muscles 6–16% and the gut usually under 10%. In Stage V and in a newly moulted female the fat contained up to 40% and the gonad was very low. If allowance were made for the small relative volume of the reproductive system the percentage activity would be still higher. In two fat females it was much lower than usual, whereas the fat contained 8 and 24%. In males the gonad was surprisingly low, being not much higher than in Stage V and the muscles were slightly higher, 12–25%. The fat fraction was unexpectedly high. It was never possible to dissect out the thin-walled vas deferens whole and its contents may have been included with the fat.

A number of females were fed for only a short time (5 min up to several hours) to see if it was possible to trace the path of the ^{32}P but in these *Calanus* the gut was usually packed full of the food and its activity was much higher than usual (15–40%). In addition, it was difficult to retain the food within the gut and it contaminated the other coverslips.

The fact that the fat in Stage V and immature females has a high value, whereas the gonad is high in mature females, confirms what had already been suspected, namely that most of the fat as it disappears goes to form the gonad.

Some ripe females were kept in a culture of radioactive *Chaetoceros*; the eggs were removed daily and their activity measured as well as that of each female at the end of the experiment. Unfortunately it was late in the year and egg-laying was poor. Those females which laid 12 to 20 eggs had 17–30% of the ^{32}P present in the eggs besides 30–45% of the ^{32}P in the reproductive system.

In another experiment in April when females were laying actively, ten were kept individually in a culture of radioactive *Dunaliella* for 2 days and on the third were transferred to inactive sea water; the eggs laid (41–158 per female) accounted for 15–50% of the total ^{32}P taken up.

In actively laying females producing perhaps several hundred eggs, the phosphorus contained in the eggs must be many times that retained in the body.

The minimum period of feeding in a radioactive culture before radioactive eggs are laid seems to be about 6–8 h. On several occasions when a female had laid twice during the night the younger eggs were more highly radioactive than the older. Thus a female which was fed for about 18 h in a radioactive culture of *Lauderia* laid two clutches of eggs (69 and 59) during this time, both of which were washed, dried, and measured for activity. The older set which must have been laid after the female had fed for about 6 h had an average of 0·5 counts per egg per min.; the younger which were laid after about 15 h of feeding had about 21 counts per egg per min.

EXCRETION OF ^{32}P IN SOLUTION

Apart from the faecal pellets produced *Calanus* must excrete the products of its metabolism. We attempted to measure the rate of this excretion so far as phosphorus was concerned by feeding a number of *Calanus* on a rich radioactive culture for about a week then putting them singly in small dishes of membrane-filtered sea water and measuring the loss of ^{32}P by the *Calanus*. Every 2 or 3 days each *Calanus* was removed, washed in three changes of water and transferred to a planchette. The water was then almost completely removed, the activity measured and the *Calanus* returned to its dish. This handling of the *Calanus* is severe treatment but it lasted only for 1 or 2 min on each occasion and did not apparently lead to any deaths. The reading of activity when the water is removed in this way is only a little below that obtained when the animal is torn up and dried, and in any case the error will remain about the same. The rate of loss is shown in Fig. 4.

It should be possible to measure the increase of ^{32}P in the water in which they are kept, and an attempt was made to do this also. There were, however, in many dishes large and unexplained losses and it is possible that some ^{32}P may have been taken up again by the *Calanus* or adsorbed on the glass or taken up by bacteria adhering to the glass. An experiment to test the last two possibilities gave, however, no definite results.

Two experiments on excretion were made, the first with twenty females fed on *Chaetoceros* when the water in the dishes was changed every time the animal was examined; the second with eight Stage V and seven females fed on *Syracosphaera*, when the water was not changed. All the Stage V which survived had moulted into females by the end of the experiment but the act of moulting had no recognizable effect on the rate of loss of ^{32}P and there was in any case very little difference between Stage V and females.

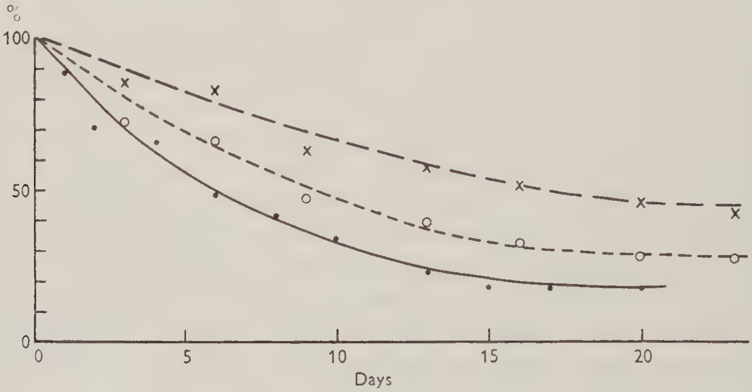

Fig. 4. Excretion of ^{32}P by starved *Calanus*. —●—, *Calanus* ♀ fed on *Chaetoceros*; —○—, *Calanus* ♀ fed on *Syracosphaera*; —×—, Stage V fed on *Syracosphaera*.

There was considerable individual variation, but the curves in Fig. 4 show that excretion is most rapid in the first week of starvation and becomes progressively less in the second and third weeks. The excretion was much higher than was expected. It was observed that unhealthy *Calanus* lost much more than healthy and in the few days before death the ^{32}P content might fall rapidly down to a figure of as little as 2 or 5 %. This seems an impossibly low figure for the body of even a moribund *Calanus*, and it is possible that the newly acquired ^{32}P is not in equilibrium throughout the body or is metabolized first. In view of the handling of the *Calanus* involved the results given should be considered as over—rather than under—estimates; these losses will also be accentuated by the starvation of the *Calanus* after a period of feeding on a rich source of phosphorus.

UPTAKE OF ^{32}P FROM SOLUTION

It has already been mentioned that *Calanus* kept in a cell-free solution of ^{32}P will become radioactive. The means by which they take in the phosphate is unknown. It may be by 'drinking' either by the mouth or by the anus, or it may be taken through the thin parts of the exoskeleton. The amount taken up varies directly with the concentration but there is a great deal of individual variation. No measurements were made of the non-radioactive P in solution, and it is possible that the uptake of ^{32}P by *Calanus* may depend on the total inorganic P present. In low concentrations (as in the filtrates of the cultures used in feeding experiments) the uptake is very low and is usually negligible when compared with the amount found in feeding *Calanus*. It is interesting, however, that after keeping females some hours in a solution of inorganic ^{32}P the eggs laid are slightly radioactive, indicating that, however the phosphate is taken in, it is rapidly distributed throughout the body.

Comparison of Feeding in Males, Females and Stage V *Calanus*

In our experiments females fed best, especially after being kept in the laboratory some days without food. A number of feeding experiments were also done with male and Stage V *Calanus*, but these were much more erratic in their feeding and very often produced no faecal pellets and took up very little ^{32}P. When they were feeding and could be compared with females it was found that the males produced usually fewer and always smaller faecal pellets and filtered less water. Stage V *Calanus*, on the other hand, on the few occasions when they fed freely, compared well with females in number and size of faecal pellets and volume of water filtered. There was no evidence that the utilization of any food organism differed with these stages of *Calanus*.

Utilization of Iodine

The utilization of phosphorus was so much higher than had been expected that it was thought advisable to test some other element which might occur in limiting concentration in the sea. Other radioactive isotopes besides ^{32}P are available for studies on feeding, and from each new information can be gained. Radioactive iodine (^{131}I) has a long enough half-life (8 days) for phytoplankton cultures to be grown and then used in feeding experiments on *Calanus*. *Lauderia borealis* was grown in Miquel culture solution with ^{131}I added. The culture grew rapidly and, after a week, had passed its period of optimum growth, although only 27% of the ^{131}I had been taken up by the diatom cells. The *Lauderia* was therefore collected on a filter and then re-suspended and used in different dilutions for a feeding experiment. Even after this treatment there was a considerable amount of ^{131}I in solution, and the *Calanus* in the filtrate from the 100% strength took up a substantial amount. Table XVIII shows the results of an experiment with female *Calanus* lasting 18 h. The total removed, faecal pellets produced, and volume filtered was remarkably small in the highest concentration as compared with the next, one-tenth of its strength, but this is not unusual. The percentage digested is very low when compared with that in experiments with ^{32}P and rises with decreasing concentration. As already mentioned, the higher figures in the 1% however may be partly due to the loss of one or two faecal pellets. From the results we may conclude that iodine in diatoms is retained by *Calanus* only to a slight degree.

A culture of *Syracosphaera* grown in Miquel with added ^{131}P took up so little of the iodine that it could not be used.

Discussion

The two most striking results which emerge from this series of experiments are the low volumes of water filtered daily by *Calanus* and the high percentage of the food digested. Both these results are at variance with generally accepted opinion. The volume of culture filtered varied from less than 1 ml. to about 30–40 ml. per day; one or two individuals exceeded this but they were exceptional. Digestion was usually over 50% and often over 90%.

In the early experiments made to determine the volume of water which a *Calanus* could filter daily (Fuller & Clarke, 1936; Fuller, 1937) volumes of 1 to 5 ml. were found. The *Calanus* were, however, kept in a very small volume of water during the experiment (3 ml. per *Calanus*) and it seemed that Gauld's later work (1951), when he kept individual *Calanus* in 100 ml. beakers, would be more reliable. He found a good deal of individual variation (42–101 ml.), but his average value was about 70 ml. a day. Harvey (1937), in the course of some short experiments on selective feeding, found values much higher even than this, reaching with *Ditylum brightwellii* rates of 168 and 240 ml. per day. It has already been pointed out that a small error in the estimation of the number of food cells may lead to a large error in the final calculation. Thus in the concentrations which Gauld used (mostly 10 to 20 cells per mm^3) an error of 1 or 2 per mm^3 in the final cell count would lead to an error of 10–50 ml. in the calculation of volume filtered. This type of error is avoided in the current experiments by measuring directly the total quantity taken up.

There is another possible source of error in the earlier work. In almost all cultures some settling out of the cells takes place unless the water is stirred (Ryther, 1954), and since *Calanus* has a habit of feeding on the bottom it will tend to get a richer supply of food than the calculated one.

Recently, Ryther (1954) has found that a substance which inhibits the feeding of *Daphnia* is produced in cultures of *Chlorella*, *Scenedesmus* and *Nitzschia*. On increasing the concentration of the food the volume filtered decreased. When a culture was in the exponential growth phase, little of this inhibitory substance was found in the water, and the *Daphnia* was affected mainly by the food ingested. In senescent cultures the inhibitory substance was present in solution also and the effect on feeding was more marked.

In the experiments on feeding *Calanus* with old cultures of *Lauderia* there was little evidence of a decrease in filtration, though the percentage digested decreased markedly. In high concentrations the volume filtered usually fell off but it seemed likely that the *Calanus* was unable to ingest the large quantity of food involved rather than that an inhibitory substance was present. There was not an inverse correlation in the lower concentrations, such as Ryther found. Although none of our experiments was designed to test for the presence of inhibitory substances, a general view (Tables XIV–XVI) does not indicate their presence in the older cultures.

A striking fact in all the experiments is the great variation between individual *Calanus* even under the same conditions. In any of the experiments some of the *Calanus* might be eating freely and others not at all, even although they were apparently as healthy and active as the rest. In different experiments on the same food species, differences were even more marked and *Calanus* filtered an average of 23·9 ml. of *Skeletonema* culture on one occasion and, on another, in a concentration little richer, only 5 ml. In Table XIX, for example, the results for *Skeletonema* indicate that more water is filtered in the higher concentrations. Yet a reference to Table XIV will show that in the experiments from which these results come, the opposite is usually true. There are, however, many factors which may influence the result which were not taken into account, e.g. age, or rather state of growth, of culture, time of year, and age or state of maturity of females.

Gauld observed that a *Calanus* can live even in a rich food culture without feeding, and this has been confirmed by our own observations. This does indicate that they are not automatic filter feeders in the sense that they must ingest all the time they are moving. Esterly (1916) has described how a food pellet collected on the filtering setae may be scattered again by a flick of the maxillipedes and this may be the usual method of rejection. The very diverse movements which Lowndes (1935) has described for the mouthparts of a *Calanus*, particularly for the second antenna, may also enable them to divert the feeding current.

The low filtration figures found make it difficult to understand how *Calanus* can maintain itself in the sea. If we accept the respiration figures found experimentally for *Calanus* (Marshall *et al.*, 1935; Clarke & Bonnet, 1939) there is apparently still a gap between the food required and the food normally present in the sea. This difficulty cannot be got over by postulating the presence of large numbers of μ-flagellates since *Calanus* seems unable to filter them off.

According to Fuller & Clarke (1936) and Fuller (1937) a *Calanus* needs to filter from 40 to 70 ml. per day to maintain itself when the phytoplankton is moderately rich. Gauld, who found daily filtrations of this order, himself showed that because of vertical migration *Calanus* will spend only part of the day feeding in the phytoplankton-rich waters. Thus the rate of filtration would have to be increased considerably for them to obtain enough.

It has been suggested by Fuller & Clarke (1936) that the fat reserve in *Calanus* might help to tide it over a period of scarcity. In Stage V *Calanus*, however, in which it is prominent, it often persists till they moult at the end of the winter and then disappears gradually. The dissection results (Table XVII) show that when Stage V moult to females, the fat may be used to build up the ovary. Digby (1954) has made the suggestion that during the winter *Calanus* are cannibals and that this accounts for the large fall in numbers over the winter.

In our experiments females were used in most cases since they fed more regularly than Stage V. Often in winter Stage V fed poorly or not at all even if starved for some days before an experiment. It may be that the metabolism and therefore the food requirement of the over-wintering Stage V *Calanus* is lower than it is during the rest of the year.

When one considers the concentration of food cells usually present in the sea, our low filtration volumes are all the more unexpected. Table XIX shows the maximum number of cells taken up by the best-feeding *Calanus* in all the experiments that were made with concentrations of food cells such as may occur in the sea. The upper limit for this was put at 16,000 per ml. Concentrations of this magnitude certainly occur only for short periods during phytoplankton outbursts, e.g. in the Oslo fjord (Braarud, 1939) and in the Norwegian Sea (Halldal, 1953), but the lowest concentrations used, down to 1 cell per ml. may be found in the sea even in winter. It will be seen that the *Calanus* can ingest up to a maximum of 600 large cells such as *Ditylum brightwellii* per day, up to 12,000 of *Syracosphaera* or *Peridinium trochoideum* and up to 50,000 of small forms such as *Platymonas*, *Cryptomonas* or *Prorocentrum triestinum*. By far the largest number was with the spring diatom *Skeletonema costatum* when nearly 400,000 were ingested. These are also very small cells but they are usually united in chains and so are more easily filtered. With still smaller forms, e.g. *Dicrateria inornata* or *Nannochloris*, the highest number ingested may be quite small (200 to 400), confirming the suggestion that these cells pass through the maxillary filter. *Chromulina* is however an exception to this for, though it is even smaller than *Nannochloris*, up to 18,000 per day were ingested in a concentration of 16,000 per ml. In low concentrations of organisms, such as might be found in the sea in winter, only a few hundred cells at most will be ingested.

While the methods used in the experiments enable us to calculate the volume filtered with some degree of certainty, the estimates of the digestion of the food taken in depend on the phosphorus alone. As is shown in Tables XIV–XVI its digestion in marine phytoplankton forms is unexpectedly high. On the other hand, the experiments with a culture labelled with ^{131}I showed that only a very small fraction of this was retained. The same must be true of other elements such as the silicon in the diatom skeleton.

The possibility that the ^{32}P may have been only loosely bound to the algal cell has already been discussed (p. 497) and Rice (1953) has shown that, under experimental conditions, little of the phosphorus can have been labile.

There were a few species in which digestion was much lower, e.g. *Chromulina*, *Dicrateria*, and possibly *Nannochloris*. In *Chlorella* viable cells were excreted and digestion could not be measured because of the breaking up of the pellets. *Bacillus globigii* spores were presumably completely indigestible, and the apparent digestion gives some measure of the spores present in the gut.

Very high filtrations are not possible in rich food cultures because faecal pellet production beyond a certain rate is not physically possible. The peristaltic movements of the gut are slow and the compacting of the faecal pellets gradual. Two *Calanus* feeding on rich *Chaetoceros* cultures produced 166 and 211 faecal pellets in 15 and 24 h respectively, i.e. a faecal pellet every 5½ or 7 min, yet even at these rates 86% was digested. These are maximum feeding rates, and as a rule faecal pellets were produced much more slowly. In concentrations such as are found in the sea (Table XIX) the highest rates were with *Chaetoceros* and with *Prorocentrum micans*, and amounted to about one every quarter of an hour; with the spring diatom *Skeletonema* the rate was about half this.

The number of cells found in one faecal pellet was counted in the experiment on choice of food already described. The maximum numbers for *Prorocentrum micans* and *Peridinium trochoideum*, which were mixed in the food culture, were 129 and 188, the averages 30 and 40. Many pellets contained 50–70 of each species. This compares well with the average figures calculated from the radioactive cultures, namely 74 and 139.

In a later experiment with *Prorocentrum micans* some of the faecal pellets were taken from three of the *Calanus* and crushed and the skeletons in them counted. The total number of cells in all the faecal pellets was then calculated and compared with the number calculated from the radioactivity of the body and faecal pellets. The number of cells per faecal pellet varied from 5 to 44 but it was not always easy to distinguish the skeletons. The agreement was reasonably good; the number of cells eaten was by radioactive measurement 1304, 1655 and 324, and by visual count 865, 1573 and 252. Although the great majority of the cells in the faecal pellets were completely empty, the percentage digestion was not so high as usual, varying from 42 to 71%.

On the whole then, when a comparison is possible the observations on faecal pellets do confirm the results obtained with radioactive cultures. Reports have often been made of faecal pellets containing many undigested cells, but when we consider the total number which a faecal pellet may represent, e.g. 1000–7000 *Skeletonema*, a few dozen undigested cells would, although apparently important, be only a small fraction of the total. There is, however, always a large amount of unidentifiable debris, and it would be desirable to know the degree of assimilation of other substances besides those containing phosphorus.

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SUMMARY

1. Cultures of diatoms and phytoflagellates were grown with radioactive phosphorus (^{32}P) present and used in feeding experiments on *Calanus*. After feeding the uptake and the volume of culture filtered was estimated by measuring the radioactivity of the body, eggs and faecal pellets.

2. The volume filtered varied from less than 1 ml. to over 40 ml. in 24 h; it is less in high concentrations of food cells but does not increase in very great dilutions.

3. *Calanus* feeds better in the dark than in the light.

4. Digestion of an old diatom culture was in some cases lower than that of a young one.

5. It was found that organisms below a size of about 10 μ could not be readily filtered off, and in such cases the volume filtered decreased with dilution of the culture.

6. Some experiments on the choice of one food rather than another show that if selection does take place it is only to a slight degree.

7. All the diatoms used could be eaten freely and most of the other algae. Digestion was poor in a few of the smallest, e.g. *Dicrateria*, *Chromulina* and *Chlorella*, but in most organisms it was unexpectedly high.

8. A high proportion of the ingested ^{32}P goes to the ovary in females and to the fat in Stage V *Calanus*. In an actively laying female a large proportion of the ^{32}P appears as eggs.

9. The excretion of ^{32}P by females initially well fed and then starved was at first rapid and slowed off after a few days.

10. Radioactive iodine (^{131}I) was used in one feeding experiment, but the utilization was low.

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TABLE I. RATE OF FAECAL PELLET PRODUCTION

Thirteen *Calanus* feeding on a mixture of *Prorocentrum micans* and *Peridinium trochoideum*

Successive ne intervals	Rate of faecal pellet production per hour													
	1	2	3	4	5	6	7	8	9	10	11	12	13	Average
5 min	0	0	*	8·0	0	8·0	0	0	0	3·8	7·5	0	4·0	2·6
0–25 min	4·0	3·8	0	0	0	6·7	3·0	3·0	3·0	0	5·7	8·2	2·7	3·1
0–25 min	2·7	2·7	3·0	2·4	2·5	7·2	5·0	2·6	2·9	11·4	5·7	5·0	7·2	4·6
½ h	2·9	2·9	2·1	1·4	0	5·5	5·3	2·0	3·3	7·1	6·5	6·3	4·2	3·8
½ h	2·0	4·0	4·1	4·1	0·7	6·1	3·5	5·2	5·8	7·1	7·1	7·2	5·4	4·8
h	3·1	4·6	3·6	5·6	0·5	5·8	3·1	6·9	5·8	7·9	7·9	6·4	7·8	5·3
7 hr	3·0	3·7	2·1	4·5	1·8	2·6	0·9	3·5	3·0	7·1	2·6	6·4	5·5	3·6

* Excreted a pellet before the first examination.

TABLE II. COMPARISON OF WASHED AND UNWASHED CELLS

		Average for 1 *Calanus* in 24 h				
Concentrations	No. *Calanus* used (♀)	No. faecal pellets	Counts/min in faecal pellets	Counts/min in body	% used	ml. filtered
460 *Lauderia*/ml. Centrifuged 4 times	4	51	2,450	21,550	89·6	32·9
320 *Lauderia*/ml. Untreated	4	28	2,500	19,090	88·6	29·9

TABLE III. SEPTEMBER 1953. UTILIZATION OF *SKELETONEMA COSTATUM*

0·13 counts/cell. 17 hr

		Eggs		Faecal pellets					
Concentration	*Calanus* (♀)	No.	(c/min)	No.	(c/min)	Body (c/min)	Total removed (c/min)	% used	ml. filtered in 24 h
0 c/min/ml., ells/mm^3	1	11	5,592	125	64,550	74,730	144,872	55·4	4·9
	2	12	4,667	120	45,850	92,510	143,027	67·9	4·8
	3	11	3,311	121	53,830	58,410	115,551	53·4	3·9
	4	0	—	80	43,860	54,620	98,480	55·5	3·3
	5	13	3,108	144	57,770	35,590	96,468	40·1	3·2
c/min/ml., ells/mm^3	1	0	—	*c.* 96	38,900	83,100	122,000	68·1	19·2
	2	0	—	*c.* 110	44,950	81,510	126,460	64·5	20·1
	3	0	—	73	29,060	30,020	59,080	50·8	8·5
	4	0	—	104	78,090	77,840	155,940	49·9	23·1
	5	23	19,070	98	75,360	67,230	161,660	53·4	23·9
c/min/ml., ells/mm^3	1	0	—	20	11,510	12,183	23,693	51·4	17·0
	2	0	—	0	—	28	28	—	—
	3	0	—	28	13,280	24,069	37,349	64·4	28·3
	4	1	215	38	14,150	24,890	39,255	64·0	30·1
	5	0	—	23	10,910	21,531	32,441	66·2	24·1
te, 5 c/min/ml.	A	0	—	0	—	16	—	—	—
	B	0	—	0	—	16	—	—	—

TABLE IV. JANUARY 1955. COMPARISON OF DIFFERENT METHODS OF MEASURING VOLUME FILTERED, USING *SKELETONEMA COSTATUM*

1·0 counts/cell. 23½ h

Initial concentration	*Calanus* (♀)	Bottle (vol. in ml.)	Faecal pellets		Body less filtrate (c/min)	Total removed (c/min)	% assimilated	ml. filtered/ 24 h	Diatom counts		Liquid counter	
			No.	(c/min)					Cells/ml. at end	ml. filtered/ 24 h (calc. by cells)	c/min/ml.	ml. filtered in 24 h
11,410 c/min/ml., 11,200 cells/ml. Liquid counter 4642 counts/min/ml.	1	70	47	40,530	120,246	160,776	74·7	16·1	—	—	3,751	15·24
	2	71	33	44,680	130,646	175,326	74·5	17·7	9,100	15·06	3,575	18·93
	3	71	23	28,460	82,006	110,466	74·2	10·6	—	—	4,225	6·83
	4	71	33	34,410	98,916	133,326	74·2	13·0	—	—	4,163	7·90
	5	72	27	26,323	79,106	105,429	75·1	10·1	—	—	4,710	—
	6	72	46	38,290	103,046	141,336	72·9	13·8	—	—	4,136	8·48
	7	73	80	70,650	176,046	246,696	71·4	26·2	7,200	32·94	3,474	21·61
	8	73	28	42,690	66,376	89,066	74·5	8·4	—	—	4,384	4·26
	9	76	c. 40	43,250	175,646	218,896	80·2	22·6	7,000	36·47	3,354	25·23
	10	75	33	40,160	117,946	158,106	74·6	15·6	—	—	4,088	9·72
Filtrate, 435 c/min/ml. Liquid counter 185 counts/min/ml.	A	38	1	5	65 } 54	—	—	—	—	—	159	—
	B	38	0	—	43 }	—	—	—	—	—	135	—

TABLE V. EFFECT OF CONCENTRATION OF *DUNALIELLA* ON FAECAL PELLET PRODUCTION

Cells/mm³	8	80	200	400	800
Faecal pellets/ *C*/24 h	11 8 } 10	59 57 } 58	69 64 } 67	81 55 } 68	82 78 } 80
Size of faecal pellets in μ	296	510	490	470	576

TABLE VI. FEBRUARY 1954. UTILIZATION OF *SYRACOSPHAERA* IN LIGHT AND DARK

16 counts/cell/min. 7.30 a.m.–5.30 p.m.

Concentration	*Calanus* (♀)	Faecal pellets		Body minus filtrate (c/min)	Total removed (c/min)	% used	ml. filtered in 24 h
		No.	(c/min)				
11,335 c/ml./min, 720 cells/ml. Dark	1	13	493	22,731	23,224	97·9	5·0
	2	28	1,423	43,902	45,325	96·9	9·9
	3	15	459	42,112	42,571	98·9	9·3
	4	13	629	31,142	31,771	98·0	6·9
	5	11	199	20,759	20,958	99·1	4·5
11,335 c/ml./min, 720 cells/ml. Light	1	12	506	30,552	31,058	98·4	6·7
	2	4	268	10,401	10,669	97·5	2·3
	3	2	252	10,933	11,185	97·7	2·4
	4	0	—	273	273	—	—
	5	4	138	7,713	7,851	98·2	1·7
Filtrate, 135 c/ml./min	A	1	0	11 } 8	—	—	—
	B	0	—	6 }	—	—	—

TABLE VII. EFFECT OF AGE OF CULTURE ON ASSIMILATION

Age of culture in days	Number of *Calanus* (♀)	Concentration used, cells/ml.	% used	Volume filtered in 24 h
5	5	155	98·4	18·2
10	8	460 320	88·1	31·4
25	6	174	66·7	14·6
32	6	1,590	58·5	8·5
5	6	1,310	73·0	19·7

TABLE VIII. SEPTEMBER 1954. UTILIZATION OF SPORES OF *BACILLUS GLOBIGII*. 18 h.

Concentration	*Calanus* (♀)	Faecal pellets No.	Faecal pellets (c/min)	Body less filtrate (c/min)	Total removed (c/min)	% used	ml. filtered in 24 h
1 %, 9520 c/ml./min	1	23	536	55	591	9·3	0·09
	2	32	344	49	393	12·5	0·07
	3	16	401	59	460	12·8	0·07
	4	19	422	51	473	10·8	0·07
	5	19	729	54	783	6·9	0·11
0·5 %	1	12	36	3	39	7·7	0
	2	26	248	24	272	8·8	0·11
	3	21	362	15	377	4·0	0·14
	4	21	188	11	199	5·5	0·05
Filtrate, 60 c/ml./min	A	0	—	2 } 1	—	—	—
	B	1	1	0			

TABLE IX. TEST FOR SELECTION BY SIZE, USING RADIOACTIVE *DICRATERIA*

Concentrations in cultures	No. *Calanus* used	Average for 1 ♀ *Calanus* in 24 h: No. faecal pellets	Counts/min in body	Counts/min in faecal pellets	Total counts/min	ml. filtered
54,000 *Dicrateria* and no *Lauderia*/ml.	5	10·7	2,613	2,030	4,657	0·57
43,020 *Dicrateria* and 1,070 *Lauderia*/ml.	5	50·8	9,856	6,046	16,050	2·43
1,070 *Lauderia* and no *Dicrateria*/ml.	5	49·7	—	—	—	—
456,000 *Dicrateria* and no *Prorocentrum micans*/ml.	5	47·4	7,204	12,847	20,051	4·73
456,000 *Dicrateria* and 108 *Prorocentrum micans*/ml.	5	104·1	7,059	18,403	25,462	6·27
108 *P. micans* and no *Dicrateria*/ml.	4	60·0	—	—	—	—

TABLE X. TEST FOR SELECTION BY SIZE, *DITYLUM–CHAETOCEROS*

Concentrations of food cells	*Calanus* (♀)	Eggs No.	Eggs (c/min)	Faecal pellets No.	Faecal pellets (c/min)	Body (less filtrate)	Total removed (c/min)	Total removed Cell vol. $\mu^3 \times 10^3$	% used	ml. filtered/ 24 h
ture 22 *Ditylum* (radio-	1	12	803	110	7,843	15,120	23,766	23·0	67·0	5·7
active) cells/ml.	2	0	—	0	—	79	79	0·7	—	—
1,500 *Chaetoceros*	3	24	284	46	1,369	9,367	11,020	10·7	87·6	2·6
cells/ml.	4	18	1,641	85	9,326	25,573	36,540	35·4	74·5	8·8
575 c/ml./min	5	11	607	80	6,808	17,128	24,543	23·8	72·3	5·8
	6	25	1,916	89	11,669	28,242	41,827	40·5	72·1	10·1
	7	4	45	15	452	7,686	8,183	7·9	94·5	1·9
rate, 950 c/ml./	A	0	—	0	—	17 } 18	—	—	—	—
n	B	11	4	3	3	19				
ture	1	34	138	1	2	882	1,022	0·7	—	—
343 *Ditylum* cells/	2	25	3,353	105	952	48,965	53,270	38·1	98·2	5·7
ml.	3	15	697	98	2,102	63,345	66,144	48·0	96·8	7·2
,500 *Chaetoceros*	4	0	—	29	129	13,236	13,365	9·7	99·0	1·5
(radioactive) cells/	5	15	10	68	1,309	58,815	60,134	43·6	97·8	6·5
ml.	6	16	1,256	90	3,994	120,675	125,925	91·3	96·8	13·9
,945 c/ml./min	7	11	42	70	1,332	38,295	39,669	28·8	96·7	4·2
	8	24	130	77	1,687	67,075	68,892	49·9	97·6	7·4
rate,	C	0	—	1	0	26 } 25	—	—	—	—
pulses/ml.	D	0	—	1	34	24				

TABLE XI. CHOICE OF FOOD IN A MIXTURE OF *PERIDINIUM TROCHOIDEUM* AND *PROROCENTRUM MICANS*

	7 *Calanus** kept 10 days in *P. trochoideum*						6 *Calanus* kept 10 days in *P. micans*					
			Skeletons excreted						Skeletons excreted			
			P. trochoideum		*P. micans*				*P. trochoideum*		*P. micans*	
Time of examination	Faecal pellets produced	Faecal pellets examined	No.	%	No.	%	Faecal pellets produced	Faecal pellets examined	No.	%	No.	%
11.46 a.m. to 12.01 p.m. (¼ h)	4	4	205	100	0	0	4	4	162	54·9	133	45
12.05–12.16 p.m. (½ h)	3	3	117	100	0	0	5	5	299	63·6	171	36
12.17–12.38 p.m. (1 h)	5	5	367	86·1	59	13·9	9	9	431	51·8	401	48
12.39–1.03 p.m. (1¼ h)	8	8	471	80·9	111	19·1	13	13	586	53·2	515	46
2.02–2.42 p.m. (2½ h)	29	17	790	59·1	548	40·9	50	17	1,774	42·7	2,334	57
3.30–4.11 p.m. (3½ h)	37	18	1,493	68·4	835	31·6	54	16	1,879	50·7	1,828	49
5.25–6.14 p.m. (5½ h)	52	19	2,861	67·9	1,354	32·1	87	18	3,205	57·7	2,351	42
10.35–11.35 a.m. (23 h)	315	21	15,258	61·1	9,725	38·9	484	18	9,671	38·8	15,259	61

* 1 ♂ included.

TABLE XII. CHOICE OF FOOD

Radioactive *Lauderia* with non-radioactive *Skeletonema*. 6 *Calanus* used in each set

	Lauderia-fed, average/*Calanus*				*Skeletonema*-fed, average/*Calanus*			
Time in mixture (h)	No. faecal pellets	Faecal pellets (c/min)	Body (c/min)	Total (c/min)	No. faecal pellets	Faecal pellets (c/min)	Body (c/min)	Total (c/min)
½	3·5	438	4,478	4,916	2·2	313	3,994	4,307
1	8·2	1,270	4,972	6,242	4·5	714	4,580	5,294
2	10·3	1,961	7,580	9,541	12·5	2,236	8,242	10,478
16*	106·7	11,047	32,855	43,790	88·3	11,021	27,594	38,615

* Count of pulses for bodies includes eggs laid.

TABLE XIII. CHOICE OF FOOD

Radioactive *Lauderia* with non-radioactive *Cryptomonas*. 6 *Calanus* used in each set

	Lauderia-fed, average/*Calanus*				*Cryptomonas*-fed, average/*Calanus*			
Time in mixture (h)	No. faecal pellets	Faecal pellets (c/min)	Body (c/min)	Total (c/min)	No. faecal pellets	Faecal pellets (c/min)	Body (c/min)	Total (c/min)
½	6·8	352	907	1,259	5·0	364	520	884
1	9·7	913	1,540	2,453	7·8	612	916	1,528
2	15·7	1,562	2,456	4,018	14·5	1,507	1,986	3,493

TABLE XIV. UTILIZATION OF DIATOMS

Species	Age of culture (days)	Concentration (cells/ml.)	*Calanus* used (♀)	Average no. faecal pellets in 24 h	% used		ml. filtered in 24 h	
					Range	Average	Range	Average
ria borealis, 29 μ av. ., 17–43 μ length	*c.* 14	627	5	77	77–88	80·4	6·6–21·2	11·5
	c. 14	63	5	40	83–90	85·6	12·0–36·4	26·5
	c. 14	6	5	8	77–95	87·8	2·7–26·2	11·3
	c. 15	645	5*	46	68–81	73·1	0·9– 9·4	4·0
	5	155	5	4	—	—	6·4–32·0	18·2
	10	320	4	27	87–91	88·6	24·3–38·0	29·9
	10	460	4	51	87–94	89·1	29·8–38·7	32·9
	25	174	6	10	66–68	66·7	11·2–20·0	14·6
	32	1,590	6	118	49–66	58·5	3–12	8·5
	5	1,310	6	113	67–79	73·0	10–33	19·7
onema costatum, n culture	13	288,000	5	183	40–68	54·5	3– 5	4·0
	13	72,000	5	144	50–68	57·4	9–24	19·0
	13	14,400	4	33	51–66	61·5	17–30	23·9
	c. 6	194,000	5	115	78–84	80·7	3– 5	3·8
	c. 6	19,400	4	48	81–87	83·8	3– 6	4·9
	c. 6	970	5	1	—	—	0·1– 1·6	—
	c. 7	770	5	3	79–96	87·0	1– 2	1·3
	11	41,700	6	75	71–89	77·6	2·4–16·8	9·8
	5	36,700	5	16	46–87	72·2	0·5– 2·3	1·2
		3,670	4	3	52–72	62·3	2·2– 2·2	2·2
	7	11,200	10	40	71–80	74·6	8·4–26·2	15·4
oceros decipiens, 1 μ diam., size range 8 μ	28	945	4	51	89–94	90·7	2–12	7·2
	28	170	5	2	—	—	0– 7	1·8
	28	17	5	3	—	—	3	2·9
	4	630	5	95	93–97	95·8	3·6–43·3	22·4
	4	63	5	31	91–98	95·3	3·8–24·7	19·9
	4	3	5	28	83–98	93·9	8·3–14·4	10·4
	8	68	5	8	97–100	98·2	1·9–10·3	6·3
	8	7	5	6	98–99	98·5	1·7– 6·2	4·1
	8	1	5	17	90–97	92·7	3·4– 8·4	5·8
	15	490	4	67	87–89	89·1	4·7– 8·1	6·1
	15	110	6	24	93–97	95·1	4·3– 8·9	6·6
	15	22	6	7	98–99	98·2	3·0– 8·6	5·0
	18	78	5	42	82–91	86·1	5·6– 8·9	7·7
	18	78	5†	12	90–98	94·4	0·2– 3·7	1·1
um brightwellii, 0 μ diam.	*c.* 20	63	4	76	85–89	87·2	1–10	7·0
	c. 20	13	5	28	90–95	91·7	2–16	9·4
	c. 20	1	4	6	87–98	93·4	2–18	11·7

* Males. † Stage V.

TABLE XV. UTILIZATION OF FLAGELLATES

Species	Age of culture (days)	Concentration (cells/ml.)	*Calanus* used (♀)	Average no. faecal pellets in 24 h	% used		ml. filtered in 24 h	
					Range	Average	Range	Average
ydomonas pulsatilla, 12 μ	3	62,000	5	85	88–90	89·0	3·0 – 5·6	4·57
	3	6,200	5	11	93–98	96·4	1·1 –31·0	11·83
	3	620	5	5	98–99	98·7	1·5 – 6·1	3·21
monas carteriaformis, 26 μ × 14–16 μ	15	41,500	6	95	61–93	77·5	2·1 – 3·1	2·59
	15	4,150	5	20	76–95	81·4	0·5 –11·6	5·15
	15	415	4	11	57–83	75·1	0·1 – 3·2	1·4
ochloris oculata, 2–4 μ	5–25	2,368,000	5	5	61–87	74·3	0	0
	5–25	119,000	5	1	27·3	—	0	0
	5–25	12,000	5	1	66·7	—	0	0
mulina pusilla, *c.* 1–3 μ	28	1,636,000	5	36	27–59	39·4	1·1 – 4·0	2·24
	28	163,600	5	15	46–68	54·2	1·4 – 1·7	1·56
	28	16,360	5	3	47–63	54·9	0·1 – 1·1	0·44
ochrysis lutheri, × 5–7 × 2 μ	7	188,000	4 *	34	50–84	71·1	0·7 – 1·2	0·45
	7	18,800	4 *	10	60–96	81·8	0·2 – 0·8	0·42
	7	1,880	4 *	9	69–97	82·0	0 – 0·7	0·22
teria inornata, 3–5·5 μ	18	2,180,000	3	59	15–41	25·9	0·2 – 0·8	0·53
	18	106,000	3	10	38–63	48·7	0·1 – 0·2	0·16
	18	10,600	3	2	62–67	64·5	0 – 0·3	0·18
	25	534,000	5	56	33–38	35·1	0·3 – 1·3	0·84
	25	279,000	4 *	50	39–59	48·5	0·2 – 0·9	0·51
cosphaera elongata, 30 × 12 μ	7	3,100	5	32	96–98	97·0	0·7 – 1·4	1·03
	7	200	5	29	98	98·0	3·6 –29·3	19·3
	7	20	5	8	98–100	98·7	3·1 –43·5	23·7
	11	720	5	38	97–99	98·1	4·5 – 9·9	7·1
	16	4,500	6	31	87–94	91·5	1·14– 1·83	1·43
	16	1,125	6	36	91–96	93·9	3·50–11·45	7·70
	16	2,800	5	15	88–99	92·6	0·5 – 3·5	1·62
	33	280	5	9	92–97	94·9	2·2 –25·6	7·35
	33	28	5	3	—	—	0·4 – 6·7	2·16
	13	575	4 *	15	97–99	98·3	0·2 – 4·1	1·80
nesium parvum, 2 × 5 μ	14	40,000	5	8	92–99	96·6	0·10– 0·47	0·37
	14	4,000	5	8	78–99	90·6	0·10– 4·22	1·77
	14	400	5	6	—	—	0·86– 1·76	1·32
tomonas sp., 27 × 6–10 μ, rage 22 × 8 μ	16	16,000	5	112	51–84	62·0	4·9 –16·7	8·7
	16	1,600	4 *	24	58–89	70·0	15·7 –32·4	22·6
	16	160	5	14	64–86	77·4	5·0 –24·7	10·8

* One omitted because it did not feed.

TABLE XVI. UTILIZATION OF DINOFLAGELLATES

Species and size	Age of culture (days)	Concentration (cells/ml.)	*Calanus* used (♀)	Average no. faecal pellets in 24 h	% used		ml. filtered in 24	
					Range	Average	Range	Aver
Prorocentrum micans	*c.* 27	68	5	69	70–86	79·3	15·3–27·6	22·
43 × 27 μ	27	540	5	54	86–96	89·6	0·5– 9·4	5·
	27	54	5	25	89–96	93·4	12·7–37·1	27·
	27	5	5	17	92–99	97·1	29·8–84·3	42·
P. triestinum 10–14 μ	*c.* 30	12,200	5	67	43–54	47·3	2·4– 4·2	3·
	c. 30	1,220	5	31	49–73	61·7	6·2–17·8	13·
	c. 31	1,020	5	39	53–65	60·2	6·9–15·6	11·
	c. 31	102	5	12	49–83	72·6	2·2–21·5	10·
Oxyrrhis marina,	14	20,400	5	52	86–92	88·7	0·6– 2·1	1·
20–45 × 15–30 μ, with	14	2,040	5	34	92–95	93·9	8·2–21·7	13·
*Nannochloris oculata**	14	204	5	8	97–99	97·9	1·5–28·1	16·
Gymnodinium vitiligo	42	7,840	5	33	86–94	88·8	5·1– 8·5	6·
6–12 μ, 14 μ long	42	784	5	15	94–97	95·8	4·8–19·2	13·
	42	78	4 †	8	97–99	98·0	1·4–23·2	9·
Gymnodinium veneficum	28	13,800	5	24	75–92	83·5	0·1– 1·3	0·
15 × 13 μ	28	1,380	5	23	90–99	94·0	0·5–11·1	5·
	28	138	5	3	99	98·6	0·3–14·5	3·
Peridinium trochoideum	32	1,750	10	81	68–85	76·4	0·7– 7·5	4·
25 × 19 μ								

* 56,000 *Nannochloris*/ml. in highest concentration.
† One which did not eat omitted.

TABLE XVII. PARTITION OF ^{32}P IN THE *CALANUS* BODY

Date	Stage of *Calanus*	Fat (%)	Carcass (%)	Reproductive system (%)	Gut (%)	Muscles (%)
23. xii. 53	M.	25·4	34·6	14·0	0·7	25·2
23. xii. 53	M.	23·3	51·9	5·0	1·1	18·7
28. xii. 53	M.	26·2	54·9	1·7	5·5	11·8
28. xii. 53	M.	37·6	42·1	1·0	1·3	17·9
28. xii. 53	M.	31·0	49·5	0·9	1·6	17·0
28. xii. 53	M.	35·1	45·2	0·6	4·6	14·4
28. xii. 53	M.	33·0	50·9	1·0	2·1	13·0
1. xii. 53	F.*	24·4	43·8	17·8	3·1	11·0
1. xii. 53	F.*	8·3	47·5	28·9	4·0	11·4
10. xii. 53	F.†	12·6	55·8	4·2	2·8	7·6
30. xi. 53	F.	—	49·5	25·2	14·4	10·7
30. xi. 53	F.	—	45·7	38·4	8·3	7·5
30. xi. 53	F.	—	41·1	40·8	7·1	11·0
4. xii. 53	F.	—	45·5	32·3	6·0	16·2
4. xii. 53	F.	—	38·5	47·1	5·2	9·2
4. xii. 53	F.	—	45·3	44·4	1·4	8·9
4. xii. 53	F.	—	48·8	44·0	1·1	6·1
10. xii. 53	V	39·8	36·7	8·2	2·3	13·0
10. xii. 53	V	25·4	48·3	11·3	6·7	8·3
18. xii. 53	V	35·3	43·5	4·9	7·6	8·7
18. xii. 53	V	34·1	48·7	2·5	10·2	4·5
18. xii. 53	V	25·7	56·0	0·4	6·6	11·3

* Fat females. † Newly moulted.

TABLE XVIII. UTILIZATION OF *LAUDERIA BOREALIS* WITH ^{131}I

18 h at 15–17° C

Concentrations	*Calanus* (♀)	Faecal pellets			Body less filtrate (c/min)	Total removed (c/min)	% used	ml. filtere in 24 h
		No.	c/min	c/faecal pellets/min				
4775 c/ml./min, 5215 cells/ml.								
100 %	1	82	3,404	42	421	3,825	11·0	1·09
	2	55	1,182	21	314	1,508*	21·6	0·46
	3	80	3,763	47	611	4,374	14·0	1·26
	4	54	1,668	31	248	1,916	13·0	0·57
	5	60	1,639	27	224	1,863	12·0	0·57
10 %	1	66	2,496	38	593	3,093†	19·3	9·04
	2	72	3,001	42	788	3,789	20·8	11·20
	3	72	3,383	47	823	4,206	19·6	12·55
	4	73	3,376	46	816	4,192	19·5	12·48
	5	89	2,540	29	628	3,168	19·8	9·38
1 %	1	3	189	63	320	509	62·9	15·46
	2	7	74	11	109	183	59·6	5·23
	3	7	227	32	104	331	31·4	9·69
	4	5	164	33	217	381	57·0	11·27
	5	3	38	13	88	126	69·8	3·66
Filtrate	A	0	—	—	103 } 122	—	—	—
3070 c/ml./min	B	6	4	1	142 }			

* Including 12 from eggs laid. † Including 4 from eggs laid.

TABLE XIX. MAXIMUM UPTAKE OF ONE *CALANUS* IN SEA-WATER CONCENTRATIONS

Species	No. cells/ml.	Counts/cell	Time of expt. (h)	No. faecal pellets/24 h	Cell equiv. per faecal pellet	Total for 24 h: No. cells taken up	Total for 24 h: ml. filtered
keletonema costatum	14,000	0·15	17	53·7	6,953	373,000	30·1
	11,200	1·02	23½	81·7	3,023	247,000	26·2
	3,670	0·28	16⅓	8·7	932	8,134	2·2
	970	0·66	18	1·3	1,443	1,924	1·6
	770	0·94	65	2·2	589	1,305	1·7
auderia borealis	627	31·0	17	112·9	11	1,198	21·2
	63	31·0	17	38·1	5	192	36·4
	6	31·0	17	7·1	2	15	26·2
	645	25·8	16	91·5	6	575	9·4*
	460	2·6	15½	52·7	290	15,310	38·7
	320	2·6	15½	37·2	275	10,230	38·0
	174	0·5	48½	11·4	279	3,176	20·0
	155	3·9	10	2·5	1,332	3,197	21·8
haetoceros decipiens	945	1·35	15½	54·2	211	11,440	11·7
	170	1·35	15½	7·7	143	1,106	6·8
	17	1·35	15½	4·6	11	53	3·2
	630	120·0	16	141·0	159	22,420	43·3
	63	120·0	16	22·5	78	1,752	31·2
	3	120·0	22¼	36·7	1	43	14·4
	490	179·0	17	81·9	46	3,784	8·1
	110	179·0	18½	22·1	34	756	8·9
	22	179·0	18½	11·7	13	146	8·6
	78	311·0	17	31·1	21	667	8·9
	68	87·0	16	19·5	33	651	10·0
	7	87·0	16	10·5	4	42	6·3
	1	87·0	21½	0	—	6	8·4
itylum brightwellii	63	385·0	18½	84·3	7	622	10·4
	13	385·0	18½	44·1	4	189	15·9
	1	385·0	18½	14·3	1	15	17·8
hlamydomonas pulsatilla	6,200	0·68	17	15·5	10,710	166,300	31·0
	620	0·68	17	5·6	653	3,685	6·1
latymonas carteriaformis	4,150	0·14	16	33·0	1,427	47,000	11·6
	415	0·14	16	22·5	60	1,350	3·2
annochloris oculata	12,000	0·03	18	2·7	97	258	0
hromulina pusilla	16,000	0·01	16¼	4·4	4,077	18,050	1·1
icrateria inornata	10,900	0·15	16	3·0	133	400	0·3
yracosphaera elongata	4,500	1·22	16½	42·2	195	8,230	1·8
	1,125	1·22	16½	40·7	298	12,140	11·5
	3,100	16·1	16	16·5	262	4,330	1·4
	200	16·1	16	49·5	104	5,125	29·1
	20	16·1	16	10·5	64	675	43·5
	720	16·0	10	67·2	101	6,800	9·9
	575	23·2	17½	24·7	97	2,324	4·1
rymnesium parvum	4,000	0·17	15¾	22·9	739	16,900	4·2
	400	0·17	15¾	9·1	78	718	1·8
yptomonas sp.	1,600	0·85	15¼	20·5	1,936	39,630	32·4
	160	0·85	15¼	14·2	252	3,567	24·7
rorocentrum micans	540	16·0	17	83·3	58	4,827	9·4
	54	16·0	17	22·6	74	1,675	37·1
	5	16·0	17	12·7	25	316	84·3
	68	111·0	9	120·0	15	1,746	27·6
. triestinum	12,200	0·84	17½	71·3	702	50,060	4·2
	1,220	0·84	17½	38·4	519	19,920	17·8
	1,020	0·76	21½	55·8	274	15,310	15·6
	102	0·76	21½	22·3	91	2,030	21·5
xyrrhis marina	2,040	0·60	18½	46·7	839	39,190	21·7
	204	0·60	18½	19·5	254	4,938	28·1
ymnodinium vitiligo	7,840	1·4	17	25·4	2,566	65,220	8·5
	784	1·4	17	79·9	1,409	13,930	19·2
	78	1·4	17	14·1	117	1,658	23·2
ymnodinium veneficum	13,800	1·54	18½	68·8	243	16,700	1·3
	1,380	1·54	18½	66·2	218	14,440	11·1
	138	1·54	18½	11·7	159	1,850	14·5

* Male *Calanus*.

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THE ROLE OF MICRO-ORGANISMS IN THE SETTLEMENT OF *OPHELIA BICORNIS* SAVIGNY

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During the course of earlier work with larvae of *Ophelia bicornis* Savigny it became increasingly clear that a major factor in stimulating these larvae to settle and metamorphose is the presence, on sand grains of suitable size, of living micro-organisms such as bacteria, in numbers neither too many nor too few (Wilson, 1954). It had been shown that an acid-cleaned sand (which is neutral or almost so) kept in sea water becomes increasingly attractive to these larvae with time. It had been admitted, however (*loc. cit.*, p. 366), that these results were derived from a comparison of experiments not designed to this end, and that further tests, planned for the purpose, were desirable.

Methods were identical with those of 1953 (Wilson, 1954), except that the relative abundance of the unmetamorphosed, metamorphosing and metamorphosed larvae in each sample as expressed in words in the tables was in 1954 based on random partial counts, giving a probable slight increase in accuracy of assessment. Unmetamorphosed larvae were not counted except when sticking to a sand grain, while metamorphosing larvae were recorded as in early, mid or late metamorphosis. These stages are not usually shown in the tables but are referred to in the text wherever the stage of metamorphosis is a matter of interest.

Fertilizations and experiments were mostly in a mixture of sea water from the International Hydrographical Station E1, and from the Clyde (by kind co-operation of the Millport Marine Station). Except when otherwise stated, all sea waters were passed through Berkefeld filter candles before use. The larvae used in the tests were always 5 days old.

THE EXPERIMENTS

In preparation for the experiments some Bullhill Bank sand which had been collected on 10 June 1952 was thoroughly cleaned in hot concentrated sulphuric acid on 21 April 1954, washed, and stored in distilled water. Such acid-cleaned sand is almost white in colour. On various dates subsequently, as noted in the description of each experiment, a quantity was removed from the distilled water, washed with filtered sea water and strewn thinly over the bottom of one or more covered glass dishes filled with filtered sea water. These were left on a bench shielded from direct light from the sky. The water in some dishes was changed a number of times at intervals of days or weeks; in others it was not changed. To a few dishes a little peptone sea water (Spencer, 1952, p. 98) was added from time to time to encourage bacteria. Further quantities of sand were similarly soaked for varying periods in unfiltered water from the laboratory circulation (tank water), sometimes mixed with mud from the bottoms of the tanks.

Each experiment was divided into two sections. Each section comprised a set of sands tested together in the same free-choice dish and often separately in conical vessels as well. The ability of the larvae to metamorphose was checked for each experiment by a control dish strewn with fresh Bullhill Bank sand. In these control dishes metamorphosis was invariably almost 100%.

Experiment 1 (*Table I*)

Section A. Four samples of acid-cleaned sand soaked in sea water for different lengths of time and one sample soaked in tank water were tested against a sample of the same acid-cleaned sand which had been stored in distilled water throughout the experiment (see Table I for dates and other details). It will be seen from the table that the longer the sand had soaked in sea water the bigger the settlement which was obtained. These soaked sands were observed to have numbers of unidentified organisms on and among their grains. Some of the organisms were almost certainly algal; bacteria would not be seen at the magnification used. All the sands were clean, but that soaked since 11 June 1954 (sample 4 in Table I, section A) had more 'algal' growths than the others. The sand from distilled water was perfectly clean.

Section B. The acid-cleaned sand samples tested here had been soaked in tank water, in one instance with the addition of tank mud, or in sea water to which peptone had been added. Some of these waters and media were changed from time to time. All the sands were discoloured by organic growths, the tank water sands being various shades of brown owing to algal growths, including diatoms. The sand from sea water with peptone medium was grey, even after washing well, and showed fluffy growths on some grains. While all these sands (after rinsing well in sea water) attracted more larvae to settle than did the sand straight from distilled water it does not appear that they were as effective as some of the cleaner sands in section A. A direct comparison with that section cannot, however, be made.

Experiment 2 (*Table II*)

Section A. It is known from earlier work that natural sands after washing and drying, or after sterilization in water at about 100° C, lose any attractive property they may have had in the fresh state, and some of them become repellent. In this series of tests acid-cleaned sands which had become attractive after soaking in sea water for several weeks (samples 2 and 5) were likewise shown to lose their acquired attractiveness by these treatments, and one of them after treatment (sample 6) seems to have become repellent. This sand from tank water and mud (sample 5), had, before washing and drying, unidentified flocculent growths on the sand grains, which were visibly more overgrown than the grains of the sand which had been soaked in clean filtered sea water (sample 2) and which appeared to be clean, except for a few small growths seen on some grains before treatment. After treatment these had disappeared, although a few dead growths were still to be seen on the grains comprising samples 6.

Section B. These tests showed that washing and drying had little or no effect on acid-cleaned sand from distilled water, so far as larval settlement is concerned, but again demonstrated that acid-cleaned sand which had acquired some degree of attractiveness by soaking in sea water lost this property after washing and drying.

Experiment 3 (*Table III*)

Section A. It was again shown that acid-cleaned sand gains in attractiveness during soaking in sea water but that this soaking has to be prolonged for several weeks to be effective. The gain after only 1 week (sample 2) was very slight, after 3 months (sample 3) very marked. Treatment with alcohol (sample 4) or formalin (sample 5) destroyed the attractiveness (the fixatives were well washed out first with fresh water, then with sea water), but there is no evidence that this particular sand had been made repellent by these treatments.

Section B. Fresh Bullhill Bank sand, collected a few days before the experiment began, was soaked for 4 days, 2 days and 1 day respectively in sea water to which peptone had been added. The peptone induced heavy growths of bacteria, especially so after 4 days, and all these sands had a pronounced smell which disappeared during rinsing in sea water before the tests were made.

From Table III it will be seen that the longer the sand had been in peptone sea water, the less attractive it was. This is even more evident if the state of the metamorphosing larvae in the conical vessels be considered. Most of those in sample 2, which had been 4 days in peptone sea water, were early and mid-metamorphosis stages, but in sample 3, which had been 2 days in peptone sea water, there was a smaller proportion of early stages and a decidedly larger proportion of late stages. In sample 4, exposed in peptone sea water for only 1 day, the metamorphosing larvae were almost without exception late stages.

Table III also shows that the fresh Bullhill Bank sand retained its attractiveness more effectively in sea water than when stored (in a stoppered jar) in its natural moist state, as collected at low tide. It is known from earlier work that the attractive property gradually diminishes when such sand is so stored.

Experiment 4 (*Table IV*)

Section A. Samples of acid-cleaned sand were soaked in sea water containing peptone, some of them for several weeks. One sample was kept sterile, in all the others bacterial growths took place and were heavy in those immersed for more than a day. Sample 2, which had been soaking longest with changes of the medium, was discoloured dark grey. This sand had also some flocculent brown growths, not present in the other sands which were relatively clean after rinsing before use although small organisms on the grains could be seen here and there.

The settlements obtained with these sands, after rinsing in sea water, are shown in Table IV. All (samples 2–5) attracted more larvae than the sand from sterile concentrated peptone sea water (sample 1), but except for sample 3 which had soaked for 10 days the settlements were not really heavy. Sample 3 produced a good settlement but the sands immersed for longer or shorter periods did not. Moreover, the metamorphosing larvae in sample 3 in the conical vessel experiment were mainly mid and late metamorphosis stages; while in all other samples, including 6, the metamorphosing larvae had a higher proportion of early stages and a smaller proportion of late. From this it would seem that acid-cleaned sand in peptone sea water increases in attractiveness for a time and then decreases (but compare Expt. 5 B). This result should also be compared with that obtained for fresh Bullhill Bank sand (Expt. 3 B) kept in peptone sea water; only a decrease in attractiveness was then recorded.

Another portion of sand from sample 2 was washed in tap water and sterilized by heating almost to boiling-point (sample 6). The attractiveness was less than the sand not so treated, but it did not become repellent. The sand grains were cleaner than the unsterilized grains in sample 2, in particular the flocculent growths were no longer visible. In the conical vessel test of this sand most of the metamorphosing larvae were relatively early stages, there being very few late stages and a smaller proportion than in sample 2.

Section B. The four sand samples tested here comprised two distinct and unrelated tests. In the first test an acid-cleaned sand soaked in sea water for about a month (Table IV B, sample 1) was compared with a similar acid-cleaned sand soaked in sea water plus 'B.B. water' (see Wilson, 1954, p. 363) for over a year (sample 2). The latter sand had been used in the 1953 series of experiments, but had been kept since then with only a single addition of fresh filtered sea water, in March 1954. Both these sands, compared with the control sample 4 from distilled water, were attractive to the larvae but that which had been soaked for a year was much more attractive than that which had been in sea water for only a month. This year-old sand was seen to have minute diatoms, other algae, a few flagellates, and apparently bacterial slimes on the grains, whereas the other sands were clean. It was not as dirty, however, as sample 2 in section A of this experiment, and was not noticeably discoloured.

The second comparison was between an acid-cleaned sand (sample 4) and similar sand (sample 3) which had been enclosed within a bolting silk envelope and buried in Salthouse Lake (Station II) sand which had previously been heated in tap water near to boiling point to ensure maximum repellence. The test was to determine if any part of the repellent property of a repellent sand can be transferred to a neutral sand in close proximity though not in contact with it. Some earlier experiments had indicated that such transference, if it occurs at all, takes place more readily when physical contact between grains is allowed (Expt. 43, Wilson, 1953*a*) and doubtfully so when the two sands are separated by bolting silk (Wilson, 1953*b*). But in these earlier experiments the sands used were fresh and unsterilized, and the attractive factor would therefore be present as well. In the present test this would not be so. The result (Table IV B) gives no indication that any part of the repellent factor was transferred. In the free-choice dish no significant difference was discernible, while comparison of the conical vessel tests suggests that sample 3 was a little more attractive than the control (sample 4). This result is explicable if it be assumed that the repellent factor consists of organic matter, which after heating is too adherent to the grains to be passed through the meshes of bolting silk. Bacteria would be able to pass through the meshes, and it is possible that the neutral sand within the silk envelope would receive bacteria from those that would multiply on this organic matter after the heated sand, heaped around the silk envelope, had been in sea water for a short time. Thus the neutral sand after receiving adherent living bacteria, and perhaps other organisms, would be slightly attractive.

Experiment 5 (*Table V*)

Section A. This was a repetition of Expt. 3 B after the sands had been kept for another 6 days. The result (see Table V) was much as before, no further marked decrease in attractiveness of the originally fresh Bullhill Bank sand having taken place, perhaps because the grains in the earlier experiment already carried as many micro-organisms as could be accommodated on their surfaces. However, the metamorphosing larvae in sample 4 from the conical vessel were not almost all late stages as they had been in the corresponding sample in Expt. 3 B, and in general there was detectable a further slight decline in attractiveness.

Section B. Some of the acid cleaned sands tested in Expt. 4 A after immersion for varying periods in peptone sea water (medium not changed) were re-tested after a further period had elapsed. The result (Table V B) is much as before (Table IV A), there not being any definite increase in attractiveness of sample 3, and especially sample 4, such as might have been expected. Sample 2 was, as before, more attractive than either. These results indicate that acid-cleaned sand kept in peptone sea water does not necessarily show a regular increase in attractiveness with time when kept in this medium, as seemed to be implied by Expt. IV A. It is not known if the organisms encouraged by the peptone were the same species for all samples and the observed difference in settlement between the various sand samples could relate to a specific difference between the organisms grown, or to their proportional abundance. It may be noted that all sands looked nearly quite clean, only a few unidentified growths being noted on some grains. The sand of sample 4 definitely had fewer visible growths on the grains than did samples 2 and 3. No such growths were seen on any grains from sample 1.

Discussion

A consideration of the 1954 experiments taken in conjunction with those of previous years makes it clear that the factor most active in inducing settlement of *Ophelia bicornis* larvae is the presence, on the sand grains, of living micro-organisms. The organisms must be neither too few nor too abundant. Presumably for a sand to attain maximum attractiveness they must be of particular species in certain relative abundance. In the experiments in which acid-cleaned sands were soaked in filtered sea water it is most unlikely that the latter requirements were even approximately fulfilled; indeed it seems probable that the species of organisms which grew under those conditions were mostly not the same and were not in the same relative abundance as those present on sand grains freshly gathered from the surface of the Bullhill Bank. None the less, after several weeks of soaking in sea water, they did impart a very considerable degree of attractiveness to acid-cleaned sand. No other explanation of the observed results seems possible.

The period needed to impart a marked attractiveness to acid-cleaned grains was very considerably shortened by adding peptone sea water to the filtered sea water in which they were immersed, but this never made the sand as attractive as did some of the prolonged soakings in filtered sea water without the addition of nutrients. Presumably the grains after immersion in the peptone sea water would become coated with micro-organisms different from those that grew slowly in pure filtered sea water, and they may well have become too abundant to attract very many larvae.

It was observed that fresh Bullhill Bank sand after immersion in peptone sea water became less attractive than before, especially after a heavy growth of bacteria had taken place. These observations support the conclusion that the quantity and quality of the living organic film on the grain surface is of first rate importance to the settling *Ophelia* larva. To the identity of the species concerned and their actual abundance there is at present no clue.

This series of studies has been another demonstration of the influence of the minutest micro-flora and fauna on the distribution and activities of larger microscopic animals and ultimately on the macro-fauna itself. They suggest that more attention could profitably be paid to the most minute living constituents of bottom deposits and their interrelations with the larger organisms subsisting in or on those soils.

The organic films left on natural sand grains after washing and drying are undoubtedly to a large extent responsible for the floatation properties of dried grains, discussed in previous papers (see especially Wilson, 1952). Less than one per cent of acid-cleaned grains stored in distilled water float when sprinkled dry on the surface of water, but during the course of the experiments recorded in this paper it was found that after some days of immersion in sea water a markedly higher percentage floated (after washing and drying). After several weeks or months the percentage of floatable grains was still higher and in one instance was over 90%. The percentage floatability of acid-cleaned sand rose steeply and more quickly during immersion in peptone sea water, and the floatation of fresh Bullhill Bank sand was also raised by immersion in peptone sea water. That flotation can be caused by electrical charges produced on the grains by rubbing is probably also true, but is likely to have little bearing on problems concerned with settlement reactions.

It seems certain that dead organic matter coating sand grains is repellent to *Ophelia bicornis* larvae and renders many sands such as Salthouse Lake sand (Stations I and II), after the silt has been washed away, distasteful to these larvae. The repellent effect is likely to be most marked if there are no living micro-organisms present as well, as when sands have been sterilized in water at 100° C, or after fixation or drying. Sands will thus vary in attractiveness and repellence according to the number and kinds of living micro-organisms, and according to the amount and quality of the non-living organic materials coating the grains. Grain size, and perhaps also shape, is another and lesser factor influencing larvae about to settle, but is not the main factor as was earlier thought (Wilson, 1948). That grade does exert some influence has often been demonstrated (Wilson, 1953*b*).

SUMMARY

Experiments in the summer of 1954 made it clear that the factor most active in inducing metamorphosis and settlement of *Ophelia bicornis* Savigny larvae is the presence on sand grains of living micro-organisms, such as bacteria, and that these should be neither too few nor too abundant. It would appear likely, and for this there is some evidence, that certain species are more effective in promoting settlement than are others, though there is at present no clue to the identities of the species concerned. Dead organisms and non-living organic matter are not attractive and may indeed be repellent, as a consideration of some of the results suggests.

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TABLE I. EXPERIMENT 1

Begun 5. vii. 54 with larvae 5 days old. Results on 7. vii. 54

Section A

Acid-cleaned Bullhill Bank sand soaked in

	(1) Distilled water since 21. iv. 54	(2) Filtered sea water, unchanged since 23. iv. 54	(3) Filtered sea water, changed 4 times since 23. iv. 54	(4) Filtered sea water, changed twice since 11. vi. 54	(5) Filtered sea water, unchanged, since 26. vi. 54	(6) Unfiltered tank water unchanged since 26. vi. 54
Settlements after 2 days in free-choice dish						
Metd	None	Many	Many	Fair number	Very few	Very few
Meting	1 or 2	Many	Fair number	Many	Very few	Few
Unmet.	Very few	Fair number	None	Good number	Several	Fair number
Settlements after 2 days in conical vessels						
Metd	Few	Very many	Very many	Fair number	—	—
Meting	Several	Many	Good number	Very many	—	—
Unmet.	Fair number	Several	Several	Good number	—	—

Section B

Acid-cleaned Bullhill Bank sand soaked in

	(1) Distilled water since 21. iv. 54	(2) Unfiltered tank water, changed 4 times since 23. iv. 54 (sand dark brown)	(3) Unfiltered tank water, unchanged since 23. iv. 54 (sand pale brown)	(4) Unfiltered tank water and mud, changed 4 times since 23. iv. 54 (sand brown)	(5) Filtered sea water containing peptone, changed 3 times since 23. iv. 54 (sand grey)
Settlements after 2 days in free-choice dish					
Metd	None	Few	Fair number	Fair number	Several
Meting	Very few	Several	Several	Few	Several
Unmet.	Several	Good number	Good number	Several	Fair number
Settlements after 2 days in conical vessels					
Metd	See A 1 above	Very few	Several	Good number	Many
Meting	—	Few	Fair number	Fair number	Many
Unmet.	—	Several	Many	Several	Few

Metd = metamorphosed; meting = metamorphosing; unmet. = unmetamorphosed.
Scale of words used to express number in Tables I–V:

Very few (sometimes also expressed in numbers 1–4)
Few
Several
Fair number
Good number
Many
Very many
Multitude
Great multitude

TABLE II. EXPERIMENT 2

Begun 10. vii. 54 with larvae 5 days old. Results on 12. vii. 54

Section A

	Acid-cleaned Bullhill Bank sand soaked in					
	(1) Distilled water since 21. iv. 54	(2) Filtered sea water, unchanged since 23. iv. 54 (sand almost white)	(3) Sand as (2) washed and dried	(4) Sand as (2), sterilized at about 100° C	(5) Unfiltered tank water and tank mud, changed 4 times since 23. iv. 54 (sand dark brown)	(6) Sand as (5), washed and dried
Settlements after 2 days in free-choice dish						
Metd	None	Many	None	None	Good number	None
Meting	None	Many	None	Very few	Good number	None
Unmet.	Fair number	Several	Fair number	Good number	Fair number	Very few
Settlements after 2 days in conical vessels						
Metd	Very few	Many	Very few	Few	Many	None
Meting	Few	Many	Few	Very few	Fair number	None
Unmet.	Fair number	Fair number	Fair number	Fair number	Fair number	Very few

Section B

	(1) Acid-cleaned Bullhill Bank sand in distilled water since 21. iv. 54	(2) Sand as (1), washed and dried	(3) Sand as (1) in filtered sea water, unchanged since 26. vi. 54	(4) Sand as (3), washed and dried
Settlement after 2 days in free-choice dish				
Metd	None	One	Several	None
Meting	None	One	Several	None
Unmet.	Fair number	Fair number	Fair number	Several
Settlements after 2 days in conical vessels				
Metd	See A 1 above	None	Several	None
Meting	—	Several	Several (early to late)	Several (all early)
Unmet.	—	Fair number	Fair number	Fair number

TABLE III. EXPERIMENT 3

Begun 21. vii. 54 with larvae 5 days old. Results on 23. vii. 54

Section A

Acid-cleaned Bullhill Bank sand soaked in

	(1) Distilled water since 21. iv. 54	(2) Filtered sea water, unchanged since 14. vii. 54	(3) Filtered sea water, changed 4 times since 23. iv. 54	(4) Sand as (3) fixed in alcohol for 1 h	(5) Sand as (3) fixed in neutral formol for 1 h
	Settlements after 2 days in free-choice dish				
Metd	None	None	Many	Very few	None
Meting	None	Very few	Many	Very few	Very few
Unmet.	Fair number	Good number	Fair number	Good number	Several
	Settlements after 2 days in conical vessels				
Metd	Very few	Several	Very many	None	Few
Meting	Fair number	Several	Good number	Fair number	Many
Unmet.	Many	Many	Several	Many	Fair number

Section B

Fresh Bullhill Bank sand (collected 15. vii. 54) soaked in

	(1) Filtered sea water since 17. vii. 54	(2) Filtered sea water containing peptone, unchanged since 17. vii. 54	(3) Filtered sea water containing peptone, unchanged since 19. vii. 54	(4) Filtered sea water containing peptone, unchanged since 20. vii. 54	(5) Fresh Bullhill Bank sand kept in natural moist state since 15. vii. 54
	Settlements after 2 days in free-choice dish				
Metd	Multitude	Few	Very few	Several	Very many
Meting	Good number	Few	Fair number	Many	Fair number
Unmet.	Several	Few	Several	Fair number	Few
	Settlements after 2 days in conical vessels				
Metd	Great multitude	Several	Good number	Multitude	Great multitude
Meting	Very few	Multitude	Great multitude	Multitude	Few
Unmet.	None	Good number	Very few	None	None

TABLE IV. EXPERIMENT 4

Begun 24. vii. 54 with larvae 5 days old. Results on 26. vii. 54

Section A

	Acid-cleaned Bullhill Bank sand soaked in					
	(1) Sterile concentrated sea water peptone since 1. v. 54	(2) Filtered sea water containing peptone, changed 5 times since 23. iv. 54	(3) Filtered sea water containing peptone, unchanged since 14. vii. 54	(4) Filtered sea water containing peptone, unchanged since 21. vii. 54	(5) Filtered sea water containing peptone, unchanged since 23. vii. 54	(6) Sand as (2) washed in tap water and sterilized at about 100° C
	Settlements after 2 days in free-choice dish					
Metd	None	Very few	Fair number	Very few	Very few	Very few
Meting	Several	Fair number	Good number	Few	Several	Few
Unmet.	Fair number	Good number	Fair number	Fair number	Fair number	Fair number
	Settlements after 2 days in conical vessels					
Metd	Very few	Very few	Many	None	Few	Very few
Meting	Fair number	Very many	Very many	Very many	Very many	Good numbe
Unmet	Several	Many	Fair number	Very many	Many	Fair number

Section B

	Acid-cleaned Bullhill Bank sand			
	(1) Soaked in filtered sea water, unchanged since 26. vi. 54	(2) Soaked in 'B.B. water' plus an equal volume of filtered sea water since 10. vii. 53. Some filtered sea water added in March 1954	(3) In bolting silk envelope in filtered sea water, amid Salthouse Lake (St. II) sand sterilized at about 100° C, since 16. vii. 54	(4) Soaked in distilled water since 21. vi. 54
	Settlements after 2 days in free-choice dish			
Metd	Fair number	Many	Very few	Very few
Meting	Fair number	Good number	Very few	Very few
Unmet.	Fair number	Many	Several	Fair number
	Settlements after 2 days in conical vessels			
Metd	Fair number	Multitude	Fair number	None
Meting	Good number	Multitude	Fair number	Fair number
Unmet.	Very many	Many	Fair number	Fair number

TABLE V. EXPERIMENT 5

Begun 27. vii. 54 with larvae 5 days old. Results on 29. vii. 54

Section A

	Fresh Bullhill Bank sand (collected 15. vii. 54) soaked in				
	(1) Filtered sea water unchanged since 17. vii. 54	(2) Filtered sea water containing peptone, unchanged since 17. vii. 54	(3) Filtered sea water containing peptone, unchanged since 19. vii. 54	(4) Filtered sea water containing peptone, unchanged since 20. vii. 54	(5) Fresh Bullhill Bank sand kept in natural moist state since 15. vii. 54
	Settlements after 2 days in free-choice dish				
Metd	Very many	Several	Few	Fair number	Very many
Meting	Fair number	Several	Few	Good number	Many
Unmet.	Very few	Few	Several	Fair number	Very few
	Settlements after 2 days in conical vessels				
Metd	Great multitude	Fair number	Fair number	Very many	Great multitude
Meting	Few	Many	Very many	Very many	Fair number
Unmet.	None	Very few	Very few	Very few	None

Section B

	Acid-cleaned Bullhill Bank sand soaked in			
	(1) Distilled water since 21. vi. 54	(2) Filtered sea water containing peptone, unchanged since 14. vii. 54	(3) Filtered sea water containing peptone, unchanged since 21. vii. 54	(4) Filtered sea water containing peptone, unchanged since 23. vii. 54
	Settlements after 2 days in free-choice dish			
Metd	Few	Fair number	Very few	None
Meting	Few	Fair number	Several	Few
Unmet.	Fair number	Good number	Good number	Fair number
	Settlements after 2 days in conical vessels			
Metd	Several	Multitude	Very many	Several
Meting	Several	Multitude	Many	Multitude
Unmet.	Very many	Few	Good number	Very many

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AN IMPROVED 'VACUUM' GRAB FOR SAMPLING THE SEA-FLOOR

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(Text-fig. 1)

The sampler to be described takes a small unstratified sample of the sea-bed, the sample being subject to a minimum of loss while being brought up. Such samples are of use in survey work, or for studies on grade size, mineralogical composition, chemical properties, or microfauna and flora of the deposits.

Although core-samples, in which the original stratification is retained, can be taken in soft muddy sediments without difficulty, it is not at all easy to take cores of sand or gravel. On such deposits an unstratified sample may be the best that can be obtained. Coarse sediments may be sampled by a dredge fitted with canvas bag, but when a small sample is required quickly, a light instrument which can be lowered vertically from the ship on a rope or hydrographic wire is needed. 'Snapper' samplers do not always take a satisfactory sample as the jaws may be prevented from closing by a pebble or shell wedged between them, much of the sample being lost while hauling up (cf. Stetson, 1938, p. 7). Stetson describes an instrument which takes a small semicircular core of sediment, the sample being sealed in the coring tube while being brought up, so preventing any loss of material. While no doubt taking good samples, the instrument is rather heavy (57 kg) and complicated in construction.

When large numbers of samples are to be taken, an 'underway' sampler as described by Pratje (1952) may be used with advantage. This requires, however, a suitably positioned winch to run out the wire as fast as the sampler descends, whereas the sampler described here can be worked either by hand or from any small winch.

The new sampler was constructed in the first place to take samples for mineralogical analysis. It is a modification of the original 'Vacuum' grab described by Hunt (1926), which is still used at Plymouth from time to time. Hunt's sampler consists of a metal cylinder containing air at atmospheric pressure, with an opening sealed by a glass plate. On reaching the sea-bottom the glass is broken by a bayonet-striker, and the resulting inflow of water to compress the air in the chamber is used to draw in a sample of sediment. Although an efficient instrument under favourable sea conditions, it was considered that the original sampler was open to considerable improvement. The most serious disadvantage is that broken glass gets mixed in with the soil sample, and the removal of the remaining fragments at the mouth of the chamber is an unpleasant operation, made more difficult by the presence of grease which is smeared round the glass to give a watertight joint. In rough weather it is difficult to obtain a satisfactory sample, probably because the sampler does not hit the bottom in an upright position. To remove the sample it is necessary to unscrew the base-plate of the chamber through a number of turns, and a quicker method of emptying out the sample was clearly desirable.

The new sampler, while retaining many of the features of the original 'Vacuum' grab, differs in having the chamber sealed by a metal plug instead of a glass plate, the shape is rather more streamlined to aid vertical descent, and the chamber can be opened more quickly to remove the sample.

DESCRIPTION OF THE SAMPLER

The sampler (Fig. 1) consists essentially of a cylindrical pressure chamber (*C*) with detachable lid (*L*) having a central opening or 'gullet' (*G*) in the bottom connected to the exterior by a downwardly directed sampling tube, consisting of two components, an upper fixed 'gullet-tube' (*T*) and a lower sliding funnel-shaped 'mouth-tube' (*M*). An extension of the gullet-tube upwards into the chamber serves as a trap-tube (*A*), which enables the sample to be retained in the chamber. The gullet is sealed by a plug (*P*) which is held in position by three retaining hooks (*H*), while the sampler is being lowered. On reaching the sea-bed, the mouth-tube slides upwards over the gullet-tube, so releasing the plug, which flies up through the trap-tube into the chamber, followed by an inrush of water and sediment collected from around the entrance to the mouth-tube.

The pressure chamber is a cylinder of 4 in. bore brass-tube, $\frac{1}{4}$ in. thick and 13 in. long, closed at the bottom by a heavy-gauge circle of metal. The lid has a rubber ring set in a lip to ensure a watertight joint, and is kept in position by a 'samson' (*S*) which can swivel sideways on bolts attached to the pressure chamber. A set screw bears down on the centre of the lid. The trap-tube (*A*) is a length of 1 in. bore pipe, 10 in. long. To the base of the pressure chamber is bolted the gullet-tube (*T*) bearing attachments for the three

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Legend to Fig. 1.

Fig. 1. Vertical section through the sampler. *A*, trap-tube; *C*, pressure chamber; *E*, stop; *G*, 'gullet'; *H*, retaining hooks (three in all, set at 120° to one another round the gullet-tube. They are pivoted between pairs of lugs attached to the gullet-tube); *L*, lid of pressure chamber; *M*, mouth-tube (stippled); *O*, collar on mouth-tube; *P*, plug; *R*, rubber washer; *S*, 'samson' securing lid; *T*, gullet-tube; *W*, brass washer; *X*—*X*, the trap-tube, washers and gullet-tube are attached to the pressure chamber by three bolts (not shown) along this axis, and opposite the retaining hooks; Y_1, slot in mouth-tube which allows limited vertical movement relative to the gullet-tube; Y_2, slot in mouth-tube to allow retaining hook to disengage completely.

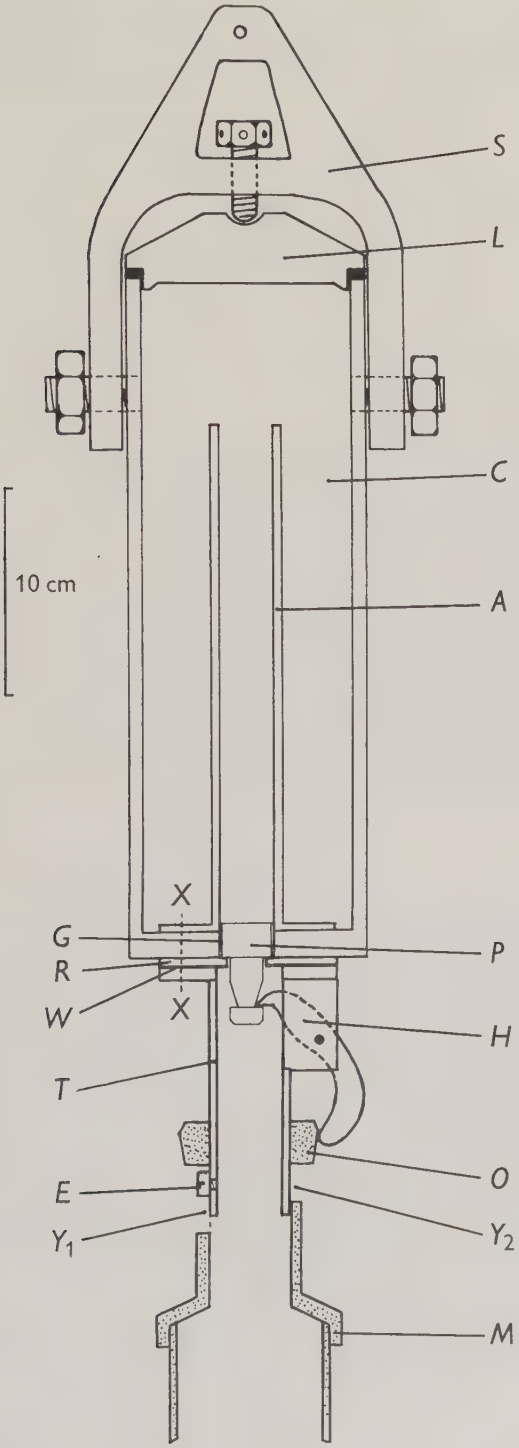
S
L
C
10 cm
A
X
G
P
R
W
X
H
T
O
E
Y₁
Y₂
M

Fig. 1.

retaining hooks (*H*), which are cut from $\frac{1}{4}$ in. brass and pivoted on $\frac{3}{16}$ in. diameter stainless steel bolts. The gaps around the retaining hooks are kept as narrow as possible to minimize leakage through the side of the gullet-tube while a sample is being taken.

The mouth-tube (*M*) slides up and down the gullet-tube, its movement being restricted by a cheese-head screw (*E*) in the latter, sliding in a longitudinal slot (Y_1) in the mouth-tube. The mouth-tube is also slotted (Y_2) to allow the retaining hooks to disengage completely. The upper part of the mouth-tube bears a tapered collar (*O*) over which the lower ends of the retaining hooks slide. When pulling down the mouth-tube to engage these hooks against the plug, the tube does not reach the limit of travel defined by the screw. Thus any wear on the surface of plug, hooks, or collar is compensated for by the mouth-tube having to be pulled down a little farther to retain the plug.

The plug (*P*) is of solid brass, and has two opposed shoulders. The retaining hooks engage on the lower one, while the upper one bears down on a $\frac{1}{8}$ in. thick rubber washer (*R*) which is bolted between the base of the pressure chamber and the gullet-tube. To prevent any possibility of the plug being pushed through the hole, a thin brass washer (*W*) of the same size and shape as the rubber one is fitted immediately below it. The size of the *lower* shoulder is such as just to pass through the hole in the rubber disk. When the sampling tube is in the 'down' position the upper lip of the plug is pressed against the surface of the rubber disk to give a watertight seal. When under water, the water pressure bearing on the rubber washer reinforces this seal.

The weight of the sampler is 30 lb. (13·6 kg).

USE OF THE SAMPLER

To prepare the sampler for use the lid is removed and the plug dropped down the trap-tube, being pushed into position by a stick. This is done with the mouth-tube in the 'up' position, so that the retaining hooks are clear of the plug. The mouth-tube is then pulled down, without exerting excessive force, to engage the retaining hooks against the plug. It is at once apparent at this stage if the plug is incorrectly seated. The lid is then replaced.

The sampler is lowered on a wire shackled to the top of the 'samson', and is brought up again as soon as it has reached the bottom.

The lid is then removed, and the contents of the pressure chamber washed into a jar. A syringe with a long plastic tube to reach down to the bottom of the chamber is useful for washing out the sample. The plug is retrieved from the sample and replaced in position for the next haul.

The samples obtained consist of about $\frac{1}{2}$ l. of sediment and $1\frac{1}{4}$ l. of water. If allowed to settle in a jar the excess water can be poured off, but for some purposes it would be sufficient to strain off the water by pouring the sample into a fine mesh sieve.

Efficiency of the Sampler

In shallow water, the volume of sediment taken increases with depth. Experiments in Cawsand Bay on a bottom of muddy sand gave the following approximate volumes of soil at different depths:

2·7 m	10 ml.
7·3 m	20, 20 ml. (2 samples)
11·0 m	100 ml.
11·9 m	35 ml.
12·8 m	100, 90, 90 ml. (3 samples)
16·5 m	250 ml. (shell gravel)

In deeper water off Plymouth (50–70 m) the sample volume is usually over 500 ml., occasionally much more. The sampler has been tested in fairly rough weather, and samples have been successfully obtained, although these are sometimes of a smaller volume than those taken under favourable conditions. Under normal sea conditions it is exceptional for the instrument not to bring up a sample.

Mr G. R. Forster has made underwater observations on the sampler in a depth of *c.* 10 m, which explain the small samples obtained in shallow water and in rough weather. On a sandy bottom the sampler drew in a crater of sand about 5 cm deep and a little wider than the mouth of the sampling tube (7·5 cm). When lowered in the normal way the sampler fell over on its side almost at once, so that the volume of sand taken should depend on the amount sucked in during the brief period when it is still upright. In very shallow water the rate of suction is such that only a small amount of sand is collected before the sampler falls on its side. In deeper water of say 30 m or more the rate of suction should be sufficient to fill the chamber before the sampler falls over, so that the sample volume should not vary greatly with depth (cf. Hunt, 1926, p. 533). When the sampler was held upright on the bottom while taking the sample a much larger volume than usual was taken, the sampling-tube penetrating for about its full length.

When the instrument was dropped free through the water from a vertical position it tended to fall with a spiral motion. If dropped from a horizontal position it did not right itself completely during the descent. Under normal working conditions the drag exerted by the wire or rope on which it is lowered would keep the sampler upright, but in rough weather the strain would not be constant and might cause the sampler to veer about during the descent, and so take a small sample or none at all.

Both the above defects might be remedied by fitting light fins or a form of drogue to the top of the instrument, and by lowering the centre of gravity of the sampler. When filled with air the 'centre of gravity' of the sampler under water is at about the mid-point of its length, so that it has no natural tendency to fall the right way up.

These modifications have not been attempted, as the sampler has so far obtained samples whenever required, and any additions would make the sampler clumsy to handle. Where samples of a quantitative nature are required, some such stabilizing attachment would be desirable.

The maximum size of particle taken is limited by the diameter of the gullet, in this instance 18 mm, so that the sampler would be ineffective on bottoms of stones or large shells, as would most other instruments except a dredge. To increase the size of the opening would necessitate strengthening the retaining hooks as the total force exerted by the water would increase with the size of the plug.

Similarly, the sampler would have to be strengthened if it were to be used at a great depth. The present sampler has been tested to a depth of 448 m with success, 1200 ml. of sand being collected in a single haul.

There may be a small loss of material via the trap-tube while the sampler is being brought up. In depths over about 45 m the air in the pressure chamber is compressed to such an extent that when upright the water-level in the chamber is above the top of the trap-tube. While hauling up, the air expands and expels water, with sediment in suspension, down to the level of the top of the trap-tube. If a flap valve were fitted to the top of the trap-tube to prevent this loss some alternative exit for the expanding air would have to be provided. Loss of fine material is considered to be small, however, and very much less than that from most other bottom samplers.

CONCLUSIONS

The new sampler has been found to be an improvement on Hunt's 'Vacuum' grab, both in ease of removal of the sample, and in the substitution of a plug for the original glass plate. On the other hand, increasing the length of the sampler has made it less stable so that it falls over almost as soon as it reaches the bottom with the result that samples in shallow water of less than about 15 m depth are not very large.

Although Hunt considered that the 'Vacuum' grab was capable of taking quantitative samples, the author considers that there must be considerable variation in the area and depth of sediment excavated, as the sampler must seldom hit the bottom truly vertically. It is therefore not suitable in its present form for making quantitative estimations related to the area of the sea-floor sampled.

The large volume of water relative to that of sediment in the sample indicates that the diameter of mouth-tube might be increased so as to obtain a larger sample.

In spite of these limitations it is considered that the sampler meets the needs for which it was designed.

I am indebted to Mr F. G. C. Ryder for his skill in making the apparatus from rough drawings, to the crew of M.L. *Gammarus* and the Master and crew of R.V. *Sarsia* for carrying out tests at sea, and to Mr G. R. Forster for underwater observations on the behaviour of the sampler. I am grateful to Mr O. D. Hunt and Dr G. A. Steven for helpful comments on the manuscript.

SUMMARY

A modified form of Hunt's 'Vacuum' grab is described for taking small samples of the bottom. The sample is subject to the minimum of loss while being hauled up, and was designed to take samples for mineralogical analysis.

The new instrument is simpler to use than the original grab, and is capable of taking samples in moderately rough weather, and in depths of 10 m to at least 448 m.

Since the area and depth sampled varies under different conditions, some modifications would be necessary for the instrument to take quantitative samples of the sea-floor.

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HEMIMYCALE COLUMELLA (BOWERBANK): A SHORT DESCRIPTION AND HISTORY OF THE SPECIES

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(Text-figs. 1–3)

These notes arise from the difficulty met with in identifying a pinkish encrusting sponge which has been frequently collected by hand from rocks at 5–10 fathoms. The sponge was at first identified, by a process of elimination, with the deep water genus *Inflatella*. Fortunately some preparations were shown to Dr Lévi at Roscoff to whom the species was at once familiar as *Stylotella columella* (Bowerbank). It was then found that this species had been described originally by Bowerbank from an Exmouth specimen, and subsequently by Topsent from both south and west coasts of France. Topsent found the unusual, if not unique, character of this sponge in possessing, besides siliceous spicules, a great abundance of calcareous granules. These granules are almost certainly the 'gemmules' described and figured by Bowerbank. De Laubenfels in 1928 collected one specimen from Plymouth Sound which he identified as *Stylotella columella*; but proposed (1932) that the species should be included in the genus *Prianos* Gray. Burton in 1934 renamed the species *Hemimycale columella*, figuring the spicules as styles. Arndt (1935) returned to *Stylotella* and retained Burton's figure of the spicules. As the styles have been found, so far, only as a small minority amongst the spicules, it has been thought worth while to provide a further description; and to discuss the differing views on the systematic position of the species.

The sponge consists of a crust, normally several millimetres thick, the surface of which is rather slimy and bears numerous shallow circular pits. These pits are very conspicuous while the sponge is alive and growing on a rock, and each consists of a group of pores. The oscules are rather infrequent, occurring on small conical papillae; they contract and close within 15 sec of a slight disturbance. The oscules are usually indistinguishable in preserved specimens. There is a distinct dermal layer or membrane which contains only scattered spicules except around the projecting rim of each pit. This rim, also slightly contractile, is supported all the way round by a single layer of numerous radially placed spicules. The main skeleton is composed of vertical fibres or bundles of spicules. The fibres often break up into smaller branches

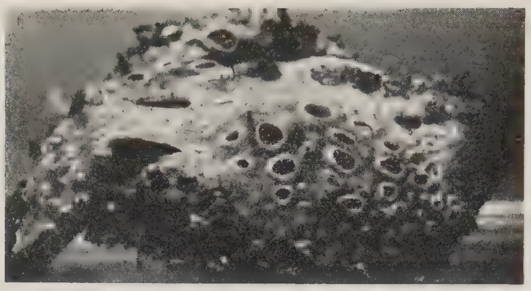

Fig. 1.

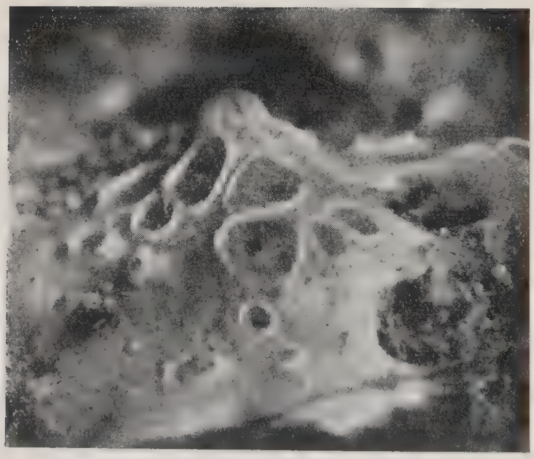

Fig. 2.

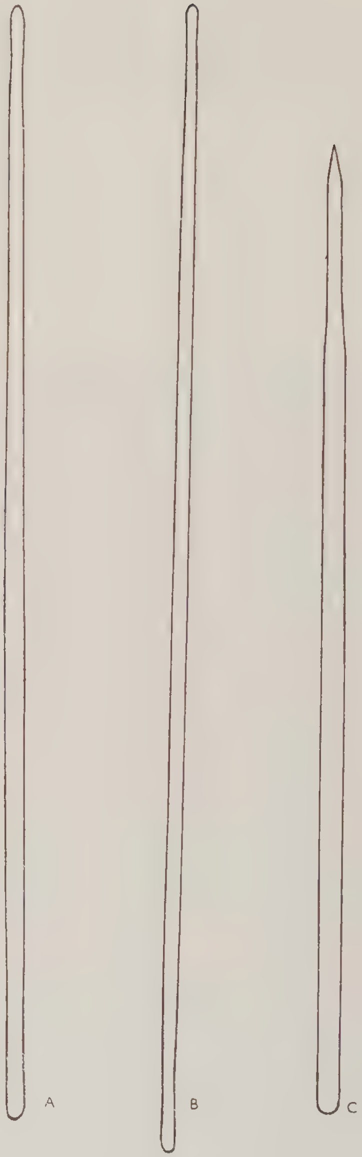

Fig 3.

Fig. 1. Living *Hemimycale columella*. Approx. natural size.

Fig. 2. Side view of the same colony, enlarged, showing an oscule.

Fig. 3. A, strongyl, 355 by 6μ; B, strongyl, 385 by 5μ; C, style, 325 by 8μ.

or give off side branches which interlock with those of neighbouring fibres. The spicules consist of strongyles with a very slight constriction near one or both ends (Fig. 3), of the following dimensions: twenty spicules measured at random ranged from 330 to 420μ with a mean length of 373μ; ten spicules had a mean breadth of 5·85μ, ranging from 5 to 6μ. Styles were also present though definitely rare: with a sample of fifteen, the mean length was 340μ and the range from 290 to 380μ. The mean breadth of five styles was 7·6μ and the range from 6·5 to 9μ.

With specimens from Naples, Topsent (1925) found the spicules sometimes entirely subtylostrongyles, but showing all gradations from subtylostrongyles to subtylostyles.

The pointed ends of the styles were often (Fig. 3) constricted rather sharply at a short distance from the point instead of tapering evenly. Topsent (1925) described the styles of three different species of *Stylotella* as having similar constrictions towards the pointed end but with a series of two or three steps in contrast with the single step of *Hemimycale columella*.

The species was first described as *Desmacidon columella* by Bowerbank (1874, p. 243); although the form of the sponge was termed massive, the arrangement and shape of the spicules agreed fairly well with that of the Plymouth specimens. The chief character which Bowerbank stressed was the gemmules. These bodies varied greatly in size and were 'exceedingly abundant, in some parts as to completely obscure the tissues beneath them'. This describes exactly the appearance of the calcareous granules in my own preparations. Although the gemmules which Bowerbank figured were considerably larger than any which I have seen, they are identical in appearance with the largest granules in a preparation of de Laubenfels' specimen. There seems no reason to doubt that Bowerbank's gemmules were also calcareous granules. Of the eleven species of *Desmacidon* described by Bowerbank only *D. fruticosum* remains in the genus.

D. columella was changed in 1891 by Topsent to *Stylinos columella*. *S. jullienni* was designated in 1892 as the type species of this genus; as this species had a skeleton composed solely of styli, the inclusion of the species *Desmacidon columella* seems at first sight hardly justifiable. Topsent, however, considered that the strongyles showed an intergrading series with the styles; this does not occur in preparations from Plymouth specimens, and, whether it takes place or not, *D. columella* seems sufficiently different from *Stylinos jullienni* and the other species of *Stylinos* for it to be included in a separate genus. Burton regards *S. jullienni* as a species of *Mycale* (verbal communication), and, therefore, since *Desmacidon columella* clearly cannot be absorbed into the genus *Mycale* as well, though, as will be shown later, it possesses some mycalid affinities, another name must be found for it.

Later, in 1894, Topsent rejected *Stylinos* as synonymous with *Stylotella* Lendenfeld (1885), whose definition was: sponges of soft texture, the spicules being styli only, singly or in bundles. *Stylotella* was revised in 1914 by Hallman who designated *S. agminata* as the type species. This species was very doubtfully assigned to the Suberitidae; while the other species described by Lendenfeld and those of Topsent were excluded from the genus, with no suggestion as to their true position. Topsent, however, continued to use *Stylotella* as an axinellid genus, noting in 1934 that Hallman's work 'leaves no doubt at all on this subject'! Moreover, Burton considers *S. agminata* to be a species of *Hymeniacidon* (verbal communication), and therefore as far as *Desmacidon columella* is concerned, he is in agreement with Hallman in rejecting *Stylotella* as the generic name. Meanwhile, in 1932 de Laubenfels had proposed to change *Stylotella columella* to *Prianos columella*, a genus which he had resurrected for Californian species. *Prianos* was introduced by Gray (1867) for *Reniera amorpha* O. Schmidt (1864). Its validity was doubted by Vosmaer 1885 and later (apparently unknown to de Laubenfels) Topsent, 1925, p. 710, found *R. amorpha* to be a true *Reniera* but synonymous with *R. cratera* O. Schmidt (1862). It is thus impossible to agree with de Laubenfels in accepting *Prianos* as a valid genus; nor in the similarity, which he suggests, between Schmidt's *Reniera* species and the species *Desmacidon columella* in having spicules 'in confusion'. For with *Reniera amorpha* Schmidt described the spicules as linked to form an irregular network by spongin, and in *Desmacidon columella* the spicules are grouped to a very large extent into branching polyspicular fibres without spongin.

Burton (1934) introduced the name *Hemimycale* for Bowerbank's *Desmacidon columella* but described the spicules as styles with occasional strongyles. Dr Burton has been kind enough to examine my preparations and now agrees as to the relative rarity of the styles. *Hemimycale* is therefore the first valid name for the species *Desmacidon columella*; but it has not been generally accepted. Arndt (1935), though using Burton's description, returned to *Stylotella* which was placed in the *Biemnidae*. Lévi (1950) notes *Stylotella columella* as fairly common in the vicinity of Roscoff, and following Topsent retains the species in the Axinellidae. Dr Lévi has since found that larvae of *Desmacidon columella* resemble those of *Mycale* species; thus as a generic for the former species, *Hemimycale* is both acceptable and appropriate.

SUMMARY

Hemimycale columella, a siliceous sponge with a simple skeleton composed chiefly of strongyles, also possesses numerous calcareous granules. The history of the sponge since its discovery by Bowerbank is reviewed and reasons given for rejecting the earlier genera in which it was placed: *Stylinos* Topsent, *Stylotella* Lendenfeld, Hallman and *Prianos* Gray. *Hemimycale* Burton is re-established.

I am much indebted to Dr Lévi for his assistance and guidance, and for permission to publish his observations on the larvae. I am also grateful to Dr M. Burton for his help, especially on the synonyms of various early genera.

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LOCAL VARIATIONS IN THE COLOUR PATTERN OF THE PRAWN *LEANDER SERRATUS* (PENNANT)

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(Text-figs. 1–2)

During investigations into the endocrinology of *Leander* (= *Palaemon*) *serratus* (Pennant) several differences in physiology (which will be reported in this *Journal* at a later date) have been found between the prawns from Plymouth and those from Roscoff on the north coast of France. These experiments involved the repeated handling and the close examination of about a thousand prawns from Roscoff and many more from Plymouth. Increasing familiarity with these animals led to the realization that the two samples could readily be distinguished by eye. It was not always possible to determine the provenance of a single individual in this way, but any group of five or six could be told at a glance. A detailed examination of the external morphology showed that the characteristic differences by which the provenance of these animals could be determined lay not in any anatomical feature but in the disposition and arrangement of the chromatophores which form the pattern on the dorsal side of the cephalothoracic shield and the anterior part of the abdomen. Great individual variations were to be seen, but differing trends of development of the patterns were found in the two populations. There was some degree of overlap between the populations, but only about 15% of the prawns lay in this region of overlap and thus failed to bear the marks of their locality; the remaining 85% could be assigned to either Roscoff or Plymouth with little difficulty. Recently I have been able to examine about forty prawns from Concarneau, on the Atlantic coast of France, and these again are mostly different from either of the other two samples.

The colour pattern of *Leander* is formed by an arrangement of varicoloured chromatophores of different types. Most of the body is covered by a groundwork of scattered, small, red and yellow, bichromatic chromatophores. Bands of deeper colour are formed by the closer massing of larger, physiologically different, bichromatic, red and yellow chromatophores which appear to contain the same pigments, but react differently to hormones. Placed singly are large, white, light-reflecting chromatophores, which also are bichromatic, possessing a relatively inconspicuous red component. Colour photographs illustrating some of these chromatophore types will be found in

Knowles (1955) and Knowles, Carlisle & Dupont-Raabe (1955). Many of the chromatophores on the legs, and some on the uropods, are monochromatic and in these parts patterns exist which are differently compounded from those on the body, but with these regions we are not now concerned. The marked differences between individuals and populations are to be found most noticeably in the dispositions of the bands of colour and the single white chromatophores on the anterior part of the body.

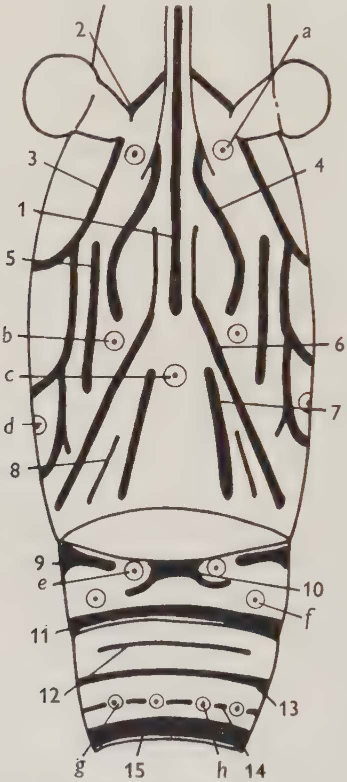

Fig. 1. Diagram of the arrangement of the bands of large chromatophores and the single white chromatophores on the dorsal side of the cephalothorax and the first two abdominal segments. 1–15, the bands of large chromatophores; *a–h*, the white chromatophores.

An arrangement of the bands and the white chromatophores which can be considered a basic pattern from which the variants diverge is represented diagrammatically in dorsal view in Fig. 1. The heavy black lines represent the bands of large, red and yellow bichromatic chromatophores, the circles the white, reflecting chromatophores, with the red component as a dot in the centre. This pattern, or something very like it, can be seen in many individuals from all three localities which have been investigated, particularly in the younger prawns. In the smaller animals it may be simplified by the omission of white chromatophores *e* and bands 9 and 10.

The main variations on this pattern take the form of reduction or suppression of certain parts or the anastomosis of bands. Asymmetry is very common: indeed, marked asymmetry seems to be more common than near symmetry.

In prawns from Plymouth one of the most constant features is the duplication, as in Fig. 2A–C, of white chromatophore *c*. This occurs in about 80% of individuals. Bands 7 from left and right frequently anastomose in front of chromatophore *c*, rarely behind. Band 1 is usually joined with either bands 6 or bands 7. The lateral horns of band 10 on the first abdominal tergite often continue round chromatophores *e* and fuse with bands 9. Band 14 and the associated chromatophores *g* and *h* may be reduced or absent. Plymouth prawns have a much greater tendency than any of the French animals to complicate the pattern by arabesques, such as are illustrated in Fig. 2B, C.

Among prawns from Roscoff only a small proportion, about 20%, shows the duplication of chromatophore *c* which is so common in prawns from Plymouth. The lateral horns of band 10 do not continue round chromatophores *e* to fuse with band 9. The main variants from the standard pattern are the suppression or great reduction in most individuals of bands 8; the frequent anastomosis of bands 7 behind chromatophore *c*, never in front of it; the reduction or interruption of bands 12 and 14, with often an increase in number of chromatophores *g* and *h* (Fig. 2E); and occasionally anastomosis of band 1 with either or both of bands 6.

In prawns from Concarneau there was a great tendency for the bands to be broken up into dotted lines, especially the narrower bands (e.g. Fig. 2G). Bands 7 usually anastomosed both before and behind chromatophore *c*, so that it came to lie in a ring. Band 1 never fused with bands 6, but did so sometimes with bands 7. The lateral horns of band 10 were often prolonged to run towards chromatophores *f* and frequently a small transverse band was developed in the mid-line between bands 10 and 11 (Fig. 2G–J). This is quite different in appearance from the slight longitudinal bar which may be present in the same position in prawns from Plymouth (Fig. 2C).

Many other individual variations may occur, but these listed above seem to be most constant in the three populations and serve best to distinguish them. Individual variations which occur in all three populations are shifts in position of chromatophores *d* so that they may not be visible in a strictly dorsal view; the appearance of an extra white chromatophore somewhere on the cephalothorax, particularly in the mid-line towards the posterior edge (Fig. 2E); or the absence of one or more of these (e.g. *b* on the right side in Fig. 2H).

It must be emphasized that these differences are described on the basis of relatively small samples of several hundred animals only (many less in the Concarneau sample). Moreover, there is the possibility that the general aspect at each locality may shift year by year. The descriptions above, and all

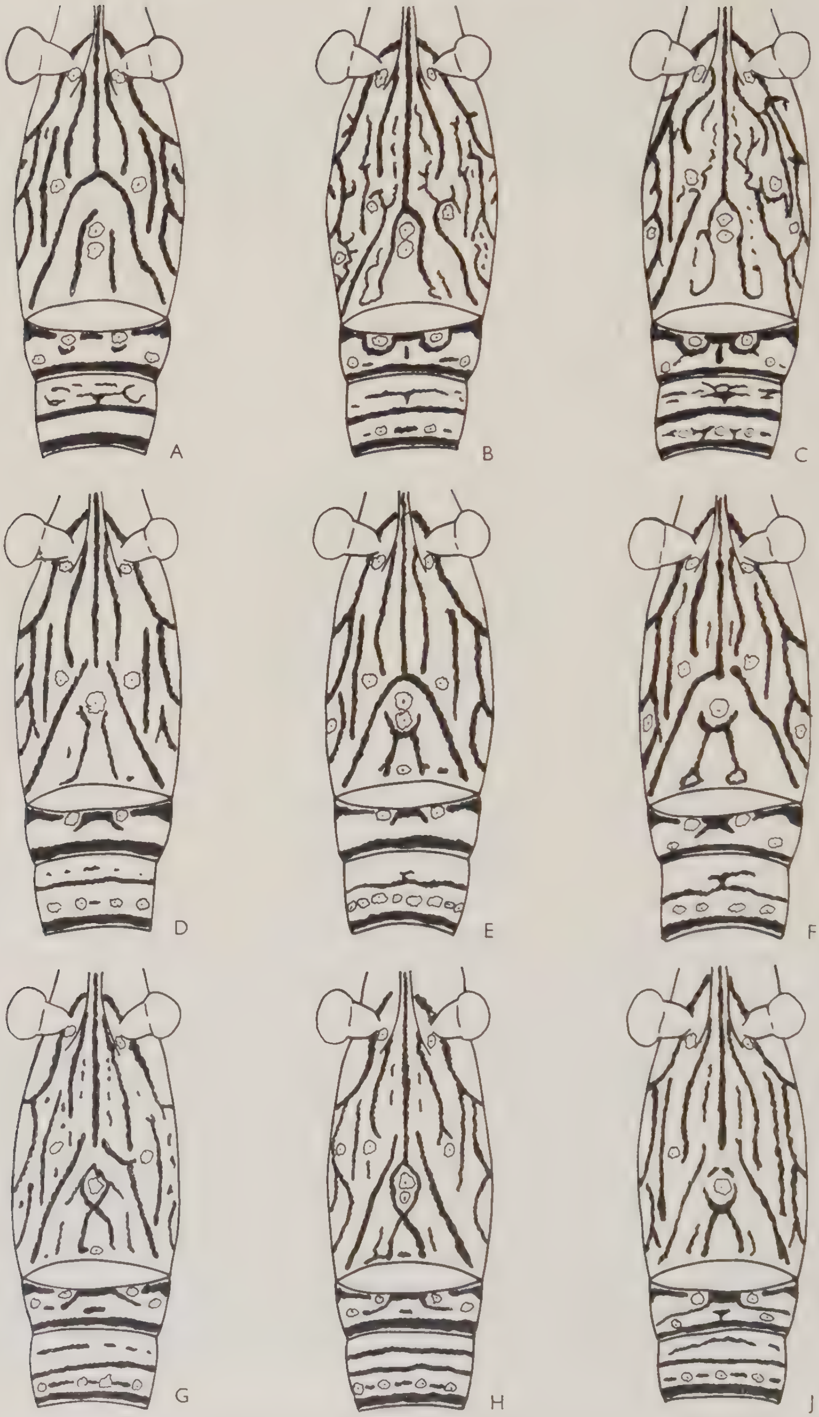

Fig. 2. Sketches in dorsal view of the patterns of colour on the cephalothorax and first two abdominal segments of female prawns of 50–55 mm overall length (rostrum to telson). A, B, C, prawns from Plymouth; D, E, F, prawns from Roscoff; G, H, J, prawns from Concarneau.

the drawings, refer particularly to females between 50 and 55 mm in length (rostrum to telson), and in smaller animals the pattern is often simplified or nearer to the standard. But even in the smallest post-larva at Plymouth the main characteristic variations can be distinguished in a proportion of individuals.

There appears to be no reason for regarding these differences as subspecific variations; it is more probable that every locality which is sufficiently isolated will show characteristic variations. The barriers which separate the three localities which I have studied are well pronounced and may form almost complete blocks in the interchange of population. They are, of course, the English Channel, and Cape Finistère and Ushant. In cross-breeding experiments with the populations from Roscoff and Plymouth there has been no depression in the fertilization rate, in the speed at which eggs are laid or in the viability of the eggs. I have reared the F_1 generation from reciprocal crosses as far as the first post-larval stage, but the numbers so far have been too few to draw any conclusions about the genetical basis of the pattern variations.

SUMMARY

Characteristic differences in the colour patterns of prawns from Roscoff, Concarneau and Plymouth are described and figured. There is no sign of any sterility between the populations from Roscoff and Plymouth.

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THE DICHOTOMOUS SPECIES OF *CODIUM* IN BRITAIN

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(Plate I and Text-figs. 1–5)

The name *Codium tomentosum* (or its nomenclatural synonym *C. dichotomum*) has been applied to dichotomous representatives of the genus in the British Isles ever since the genus was established by Stackhouse in 1797. The purpose of this paper is twofold: to show that three distinct species are involved; and to clarify the nomenclature.

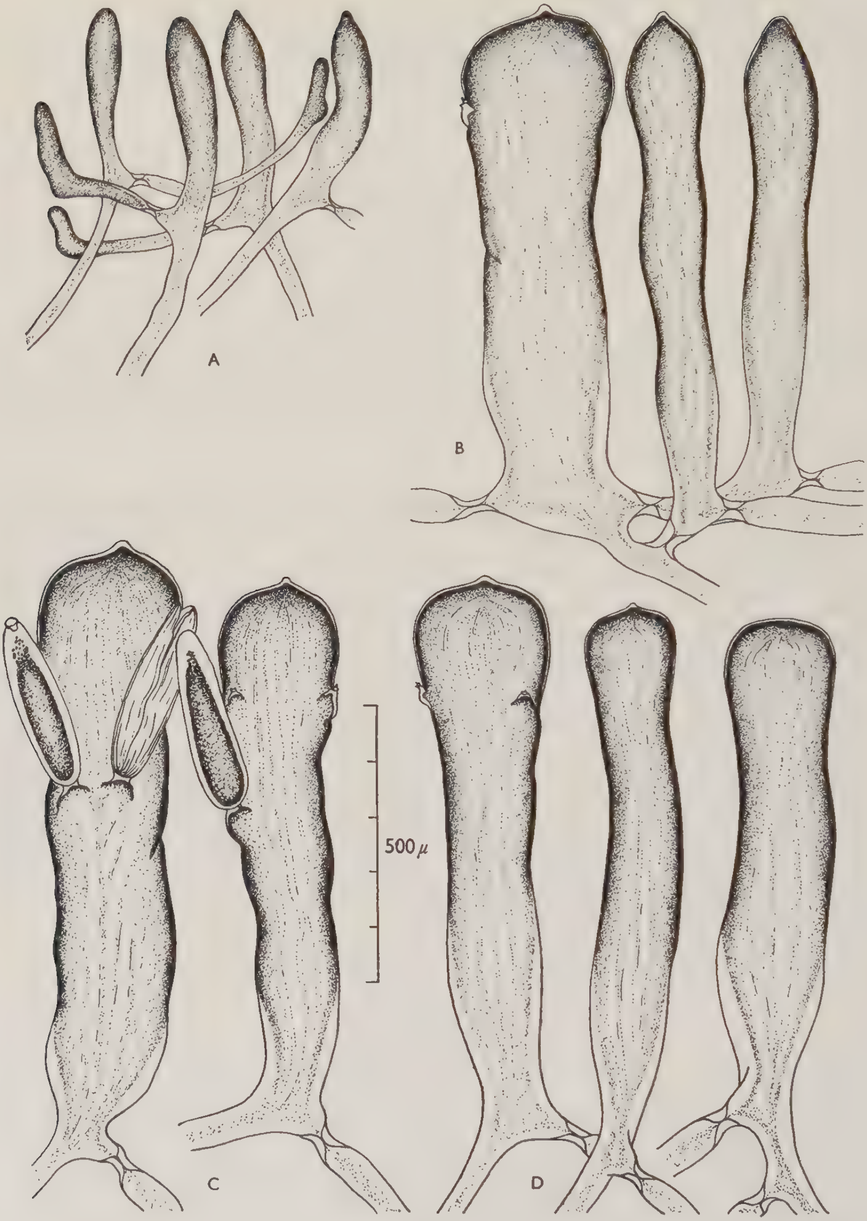
I am grateful to the directors and curators of the following herbaria for the loan of specimens: British Museum (BM); Royal Botanic Garden, Edinburgh (E); Farlow Herbarium, Harvard University (FH); University of Glasgow (GL); Royal Botanic Gardens, Kew (K); Rijksherbarium, Leiden (L); Linnean Society of London (LINN); University of Michigan (MICH); New York Botanical Garden (NY); University of Oxford (OXF); Muséum National d'Histoire Naturelle, Laboratoire de Cryptogamie, Paris (PC); Marine Biological Association, Plymouth (PL); Naturhistoriska Riksmuseet, Stockholm (S); Trinity College, Dublin (TCD); University of California, Berkeley (UC); United States National Museum (US). Liquid-preserved and dried material, which has been placed in the Herbarium of the University of California, was kindly provided by Dr Sheila Lodge, Dr Betty Moss, Dr Mary Parke, Dr Máirín de Valéra, and Dr M. A. Wilson. Special thanks are due to Dr Mary Parke for her sustained interest, encouragement, and help in this study.

Codium fragile (Sur.) Hariot subsp. *atlanticum* (Cotton) Silva, n.comb.

(Text-fig. 1)

Codium mucronatum J. Ag. var. *atlanticum* Cotton, 1912, p. 114, pl. VII; pl. VIII, figs. 3–5.
C. fragile (Sur.) Hariot var. *typicum* O. C. Schmidt subvar. *atlanticum* (Cotton) O. C. Schmidt, 1923, p. 47.
C. tomentosum Stackh. var. *atlanticum* (Cotton) Lily Newton, 1931, p. 106.
C. fragile (Sur.) Hariot f. *atlanticum* (Cotton) Levring, 1937, p. 34.
C. atlanticum (Cotton) De Valéra, 1939, p. 12.

Thallus composed of one to several erect robust fronds arising from broad, spongy, basal disk; fronds 15–25 (–50) cm high, dichotomously branched (to 9 orders), dichotomies at times telescoped to give fastigiate appearance;



Text-fig. 1. *Codium fragile* subsp. *atlanticum*. A, utricles from tip; B, utricles from base; C, D, utricles from middle of frond. A, B, D, Carna, *de Valéra*; C, Port St Mary, *Lodge*.

branches terete (at times flattened at base), tapering from base (*c.* 10 mm diam.) to apex (2·5–4 mm diam.). Utricles irregularly cylindrical or clavate, (130 –) 170– 330μ max. diam., 780–1100 (– 1200) μ long, usually 3–5 × long as broad; apices slightly rounded, often terminating in a blunt (or occasionally sharp) point or umbo; wall of utricle *c.* 1·5μ thick, thicker at apex (– 12μ) and often prolonging point into mucron 10–14μ long. Hairs (or hair scars) common, one or two per utricle, borne 130–200μ below apex. Gametangia ovoid, oblong, or fusiform, 80–130μ diam., 260–400μ long, one or two per utricle, each borne on protuberance near middle of utricle. Medullary filaments mostly 28–68μ diam.

Type. Rock pool, Roonah Point (opposite Clare Island), Co. Mayo, Eire, June 1910, *A. D. Cotton* (K).

Geographic distribution. Western Ireland northward to western Scotland, Orkney Is., Isle of Man, Northumberland, Dorset, Norway.

Representative specimens examined. **Ireland**. CLARE: Kilkee, Oct. 1897, *E. George* (BM). GALWAY: Muigh Inis, Carna, Oct. 1943, *M. de Valéra.* MAYO: Kinnacorra, Clare I., Aug. 1911, *A. D. Cotton* (BM); Roonah Point, June 1910, *A. D. Cotton* (K) (type). SLIGO: Sligo Bay, July 1850, *Hennedy* (GL). DONEGAL: Bundoran, July 1850, *W. H. Harvey* (TCD). ANTRIM: Portrush, Oct. 1858, *Hennedy* (GL); Giants Causeway, May 1910, *A. D. Cotton* (K); Larne, Aug. 1865, *Mrs C. A. Johnson* (BM). DOWN: Tara Point, Ards Peninsula, Aug. 1953, *C. I. Dickinson* (K). **Isle of Man**. Bradda Head, Port Erin, April 1912, *R. J. Harvey-Gibson* (K); Port St Mary, May 1950, *S. Lodge.* **Scotland**. BUTE: Eilans, Cumbrae, Aug. 1891, *E. Batters* (BM); Keppel, Cumbrae, Apr. 1955, *E. Conway* (GL). ARGYLL: Iona, June 1826, *R. K. Greville* (E). ORKNEY: South Ronaldshay, Aug. 1891, *G. W. Traill* (BM); Pharay, Aug. 1880, *Boswell* (E); Westray, Aug. 1845 (TCD). INVERNESS: Mallaig, Aug. 1949, *E. Conway* (GL). **England**. NORTHUMBERLAND: Berwick-upon-Tweed, Oct. 1949, *B. Moss*; Farne Is., Nov. 1952, *B. Moss.* DORSET: Swanage, Aug. 1894, *E. Batters* (BM).

Codium fragile (Sur.) Hariot subsp. *tomentosoides* (van Goor) Silva, n.comb.

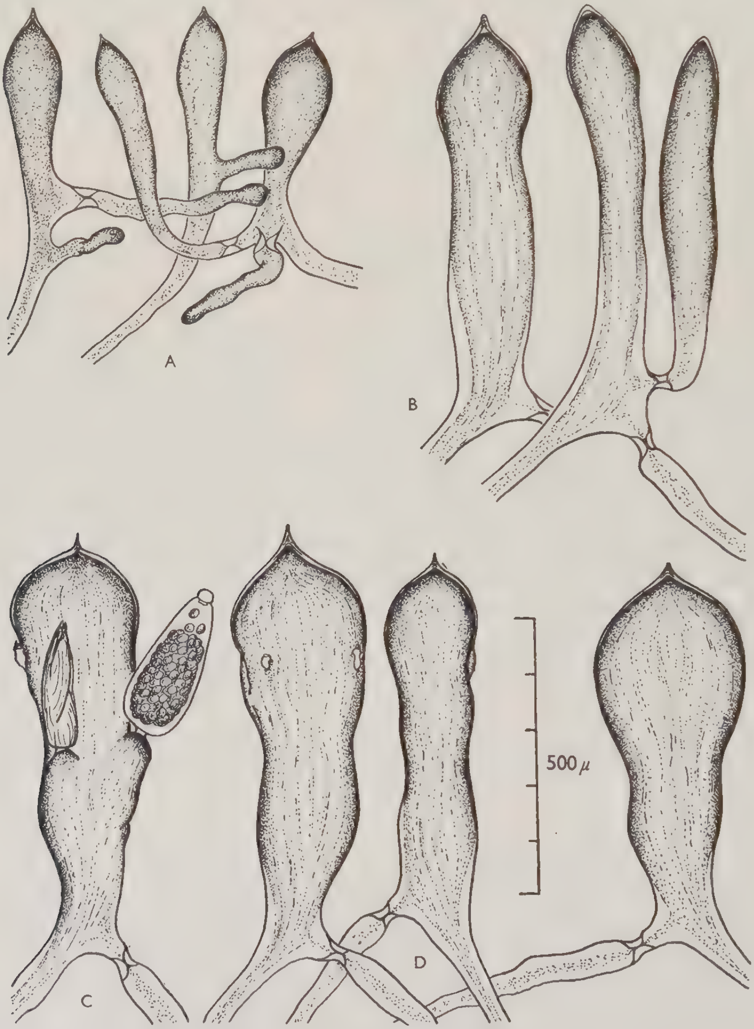
(Text-figs. 2, 3)

Codium mucronatum J. Ag. var. *tomentosoides* van Goor, 1923, p. 134, fig. 1*c.*

Thallus as in *C. fragile* subsp. *atlanticum.* Utricles irregularly cylindrical or more often clavate, frequently with broad constriction at or just below middle, (105 –) 165–325 (– 400) μ max. diam., 550–1050μ long, usually 2·5–5·5 × long as broad; apices rounded-apiculate; wall of utricle *c.*1·5μ thick, thicker at apex (– 12μ), prolonging point into mucron up to 68μ long. Hairs (or hair scars) common, one or two per utricle, borne 160–260μ below apex. Gametangia ovoid, oblong, or fusiform, 72–92μ diam., 260–330μ long, one or two per utricle, each borne on protuberance near middle of utricle (410–560 μ below apex.) Medullary filaments mostly 26–68 μ diam.

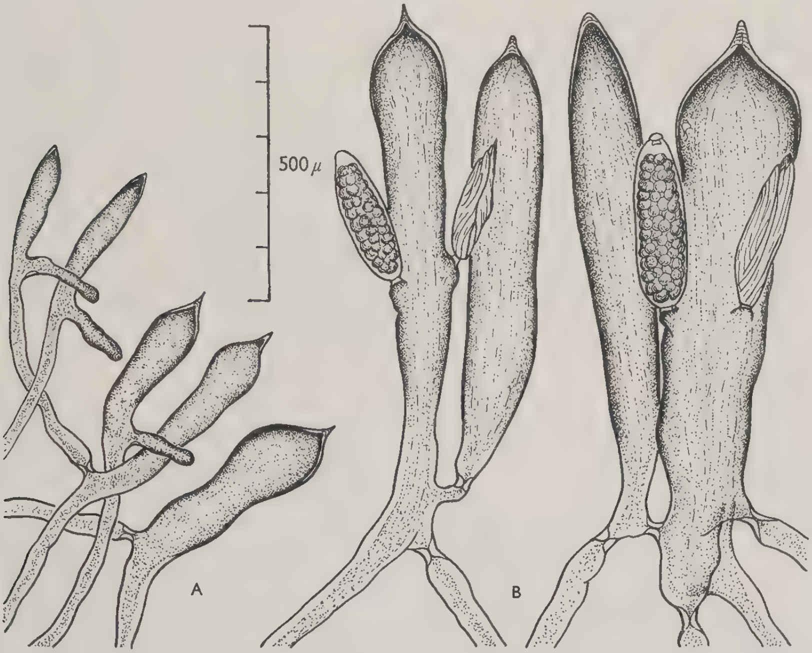
Type. Huisduinen, Netherlands, 1900, *Mrs J. L. Redeke-Hoek* (specimen not located). In the absence of the holotype, I have selected as neotype a specimen from Helder, Netherlands, annotated by van Goor, in the possession of Prof. J. Heimans, Hugo de Vries Laboratorium, Amsterdam.

Geographic distribution. Southern England, Scotland (Argyll), Ireland (Cork) France, Netherlands, Scandinavia.



Text-fig. 2. *Codium fragile* subsp. *tomentosoides.* A, utricles from tip; B, utricles from base; C, D, utricles from middle of frond. A, D, Steer Point, *Hunt*; B, C, Schouwen, Holland, Aug. 1938, *N. J. N. Kamp* (L).

Representative specimens examined. **England.** CORNWALL: Trevone, Aug. 1953, open pool in current, mid-littoral, *M. A. Wilson.* DEVON: Bantham, July 1953, edge of large deep pool, *M. A. Wilson*; Yealm Estuary, near Steer Point, Nov. 1939, on oyster, *O. D. Hunt* (BM). DORSET: Kimmeridge Ledges, Apr. 1955, *C. I. Dickinson* (K). SUSSEX: Elmer Sands, 3 miles E. of Bognor, Aug. 1954, cast up, *R. W. J. Keag* (K). **Scotland.** ARGYLL: Loch Sween, June 1953, *E. Conway* (GL). **Ireland.** CORK: Lough Ine, July 1950, *S. Lodge.*



Text-fig. 3. *Codium fragile* subsp. *tomentosoides.* Lough Ine, *Lodge.* A, utricles from tip; B, utricles from middle of frond.

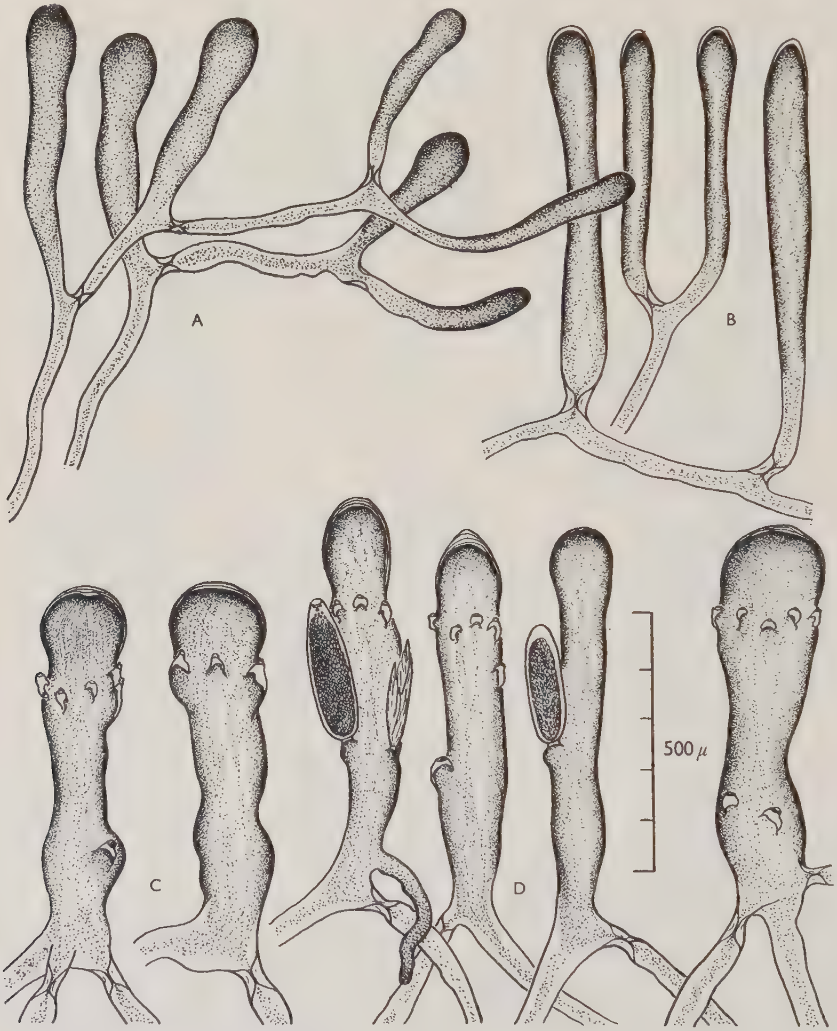
Codium tomentosum Stackhouse

(Plate I, fig. 1; Text-fig. 4)

Codium tomentosum Stackhouse 1797, p. xxiv. 1795, p. 21 (sub *Fucus tomentosus* Hudson excl. syn. Linn.).
Fucus tomentosus Hudson var. *marginifer* Turner, 1811, p. 1.

Thallus composed of one to several erect fronds arising from broad, spongy, basal disk; fronds 20–40 cm high in pools, 45–60 (–90) cm high in deep water, dichotomously branched (to nine orders), dichotomies at times telescoped to give subfastigiate appearance, at other times distant (internodes

7–8 cm long), rarely proliferous; branches mostly terete but frequently flattened at dichotomies, 3–5 mm diam. in lower parts, to 8 mm at dichotomies, attenuating to 1·5–2 mm at apices, terminal segments often long (5–11 cm). Utricles cylindrical or slightly clavate, often with hip-like enlargement near base, (65 –) 100–200 (– 275) μ max. diam., (420 –) 500–800 (– 1050) μ long, (3 –) 4–7 (– 9) × long as broad; apices rounded or tending to be pointed; apical wall usually moderately thickened (– 44 μ), lamellate, at times introrsely umbonate and/or extrorsely bluntly pointed. Hairs (or hair



Text-fig. 4. *Codium tomentosum*. A, utricles from tip; B, utricles from base; C, D, utricles from middle of frond. A–C, Carna, *de Valéra*; D, Wembury, *Ryder*.

scars) common, numerous, borne at conspicuous distance below apex (130–235μ), forming collar below broadest part of utricle. Gametangia ovoid, oblong, or fusiform, 50–110μ diam., 165–340μ long, one to four per utricle, each borne on prominent protuberance one-half to two-thirds distance below apex. Medullary filaments mostly 20–50μ diam.

Type. 'On the Devonshire and Cornwall Coasts: on the Long Rock between Marazion and Pensance[1] plentiful. Hon. Mr. Wenman' (specimen not located). In the absence of the holotype, I have selected as neotype a specimen in the Linnean Society collected at Acton Castle, presumably by Stackhouse.

Geographic distribution. Western Ireland, Orkney Islands, southern England, Channel Islands, Netherlands southward to Morocco, Azores Islands, Algeria.

Representative specimens examined. **Ireland**. CORK: Bantry Bay, July 1808, *Miss Hutchins* (K) (type of *Fucus tomentosus* var. *marginifer* Turner). CLARE: Kilkee, July 1833, *W. H. Harvey* (TCD, K, NY). GALWAY: Muigh Inis, Carna, Oct. 1943, *M. de Valéra*; Renvyle Point, Jan. 1831, *R. J. Shuttleworth* (BM). MAYO: Clare Island, Aug. 1911, *A. D. Cotton* (BM, K, NY). SLIGO: Sligo Bay, July 1850, *Hennedy* (GL). DONEGAL: Malin Head, July 1897, *M. R. Clarke* (FH, NY). ANTRIM: Ballycastle, 1797–98, *R. Brown* (BM). **Scotland**. ORKNEY ISLANDS, *J. H. Pollexfen* (BM). **England**. CORNWALL: St Mary's, Scilly Islands, Sept. 1953, *E. Conway* (GL); Acton Castle, *Stackhouse* (LINN) (neotype); King's Cove, June 1799, *D. Turner* (BM, K); Mullion Cove, Jan. 1932, *Lyle* (BM); Gerrans Bay, *E. M. Holmes* (BM); West Looe, Nov. 1952, *M. Parke* (PL). DEVON: Wembury, Oct. 1953, *F. G. C. Ryder* (PL, UC); Salcombe, May 1953, *D. Ballantine* (PL); Torquay, Wyatt's *Algae Danmonienses* no. 35 (BM, E, K, L, NY, TCD, UC); Exmouth, 1864, *Mrs C. A. Johnson* (BM). DORSET: Weymouth, Sept. 1892, *E. Batters* (BM); Swanage, Aug. 1894, *E. Batters* (BM). SUSSEX: Brighton, July 1889, *Miss Palmer* (BM). **Channel Islands**. Guernsey, Aug. 1840, *Lady Mansell* (BM).

A plant of *C. tomentosum* in which the dichotomous branches bear numerous simple and forked proliferations was described by Turner as a variety (*marginifer*) of *Fucus tomentosus*. This proliferous condition is rare in *Codium tomentosum* compared to *C. vermilara*.

Codium vermilara (Olivi) Delle Chiaje

(Plate I, fig. 2; Text-fig. 5)

Lamarckia vermilara Olivi, 1792, p. 258, pl. VII.
Myrsidrum vermilara (Olivi) Rafinesque, 1810, p. 98.
Codium vermilara (Olivi) Delle Chiaje, 1829, p. 14.

Thallus composed of one to several erect fronds arising from small, spongy, basal disk; fronds 10–45 cm high, closely dichotomously branched (to 10 orders), often irregularly so, branches frequently bearing simple or forked proliferations; branches terete, tapering from *c.* 4 mm at base to 1·5–2 mm

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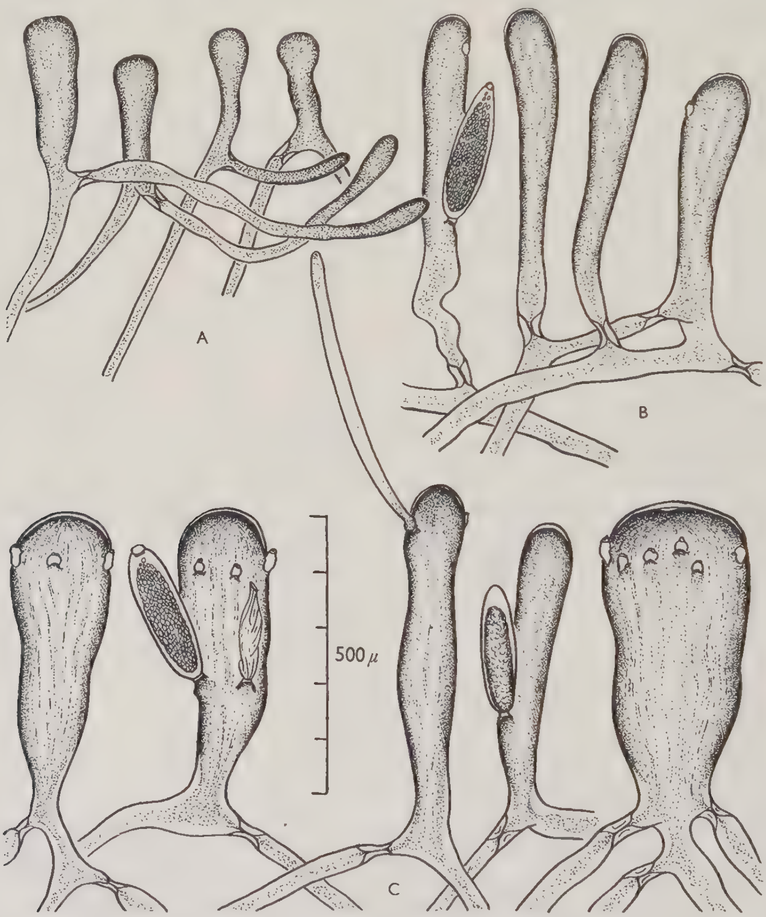
[1] Old spelling of Penzance (ED.).

at tips, terminal segments usually short. Utricles clavate to pyriform, (65–) 90–240 (–340) μ diam., (330–) 400–650 (–1000) μ long, 1·5–4·5 (–7) × long as broad; apices rounded; apical wall slightly to moderately thickened, 5 to 15 (–50) μ, lamellate. Hairs (or hair scars) common, numerous, borne on shoulder of utricle 60–110 μ below apex. Gametangia oblong to fusiform, (52–) 60–80 (–96) μ diam., 200–290 μ long, one to four per utricle, each borne on protuberance one-half to two-thirds distance below apex. Medullary filaments mostly 18–45 μ diam.

Type. No specimens mentioned in original description. I have selected as neotype a specimen in the Herbarium of the University of California at Berkeley distributed as Flora Exsiccata Austro-Hungarica no. 794 under the name *C. tomentosum*, collected by Zay at Portoré, Croatia.



Text-fig. 5. *Codium vermilara*. Wembury, *Ryder*. A, utricles from tip; B, utricles from base; C, utricles from middle of frond.

Geographic distribution. Ireland (Galway, Antrim), Scotland (Argyll), southern England, Channel Islands, Norway, France southward to Morocco, Mediterranean, including Adriatic.

Representative specimens examined. **Ireland.** GALWAY: Galway, *McCalla* (TCD). ANTRIM: Portrush, 1835, *D. Moore* (K, TCD); Cushendall, *W. H. Harvey* (BM). **Scotland.** ARGYLL: Portnahaven, Islay, 1826, *J. Chalmers* (BM, GL, K). **England.** DEVON (north coast): Ilfracombe, Oct. 1807, *R. Hare* (BM). CORNWALL: Penzance, *M. P. Merrifield* (BM); St Michael's Mount, June 1799, *D. Turner* (K); Acton Castle, June 1798, *Stackhouse* (LINN); Falmouth, *F. W. Smith* (BM); Gerrans Bay, *E. M. Holmes* (BM). DEVON (south coast): Wembury, Oct. 1953, *F. G. C. Ryder* (PL, UC), Salcombe, July 1941, *M. Parke* (PL); Harbrick, Torbay, March 1833, *Mrs Griffiths* (BM); Torquay, *Lyon* (BM); Exmouth, *Miss Baker* (K); Sidmouth, Nov. 1829 (TCD). DORSET: Weymouth, Dec. 1911, *A. D. Cotton* (K); Swanage, *E. M. Holmes* (US). HANTS: West Cowes, Isle of Wight, *Count Pouliatine* (BM). **Channel Islands**: Jersey, *F. P. Girdlestone* (MICH).

DISCUSSION

The presence in the British Isles of a *Codium* with mucronate utricles was first demonstrated by Cotton (1912), who described *C. mucronatum* var. *atlanticum*. This entity is herein referred to *C. fragile* in accordance with the general agreement that the latter is conspecific with *C. mucronatum*. Taxonomic treatment of *C. fragile* is complicated by the following factors: (i) the species in the broadest sense may be considered a complex assemblage of populations and series of populations, some of which are morphologically homogeneous and others heterogeneous; (ii) evidence is present for hybridization or gene flow between plateaus of morphological uniformity; (iii) the species has by far the most extensive geographic distribution of any *Codium*, occurring on all continents; (iv) unusual physiological adaptability is implied by the variety of habitats, from the sublittoral to the midlittoral, from surf-swept rocks to quiet shell-mud flats; (v) the species has the vigour and adaptability of a weed and is spreading rapidly in such places as Scandinavia, France and Spain. A complete consideration of *C. fragile* will be presented in a forthcoming monograph of the genus. For the present account it is sufficient to state that in the British Isles there are two series of populations of mucronate *Codium*, each relatively uniform morphologically, which I consider best treated as subspecies of *C. fragile*.

The present centres of distribution of *C. fragile* are the Pacific and Subantarctic oceans. By far the greatest morphological heterogeneity occurs in Japan. In fact, almost all subspecies (geographically discrete series of morphologically uniform populations) can be matched by one or more Japanese collections. It is possible that *C. fragile* subsp. *atlanticum* was introduced from the Pacific within historical times. According to Cotton (1912, p. 116) it was collected at Bantry Bay by Miss Hutchins (*c.* 1808?). Another early collection is one made by Greville on the island of Iona in 1826. Chalmers (*Algae Scoticae Exsicc.* no. 13, 1826) stated that *Codium* was unknown in Scotland before 1825, when it was discovered on Iona by Berkeley. This subspecies seems to be spreading: Cotton (p. 119) noted that it probably was established on the Isle of Man in 1900, and the presently cited Northumberland and Swanage collections are the first published English records. (The Swanage record seems out of range and some doubt is thus raised as to the correctness of the locality.) The earliest known collection from Norway is dated 1918 (Hussay near Molde, *N. Wille*, S).

Codium fragile subsp. *tomentosoides* seems definitely of very recent occurrence in Europe. It was first collected in Holland in 1900 (van Goor, 1923, p. 133), Denmark in 1920 (Rosenvinge, 1920, p. 131), Sweden in 1938 (Brandholmen near Långő, Bohuslän, *C. Engblad*, S), Norway in 1952 (Bratvaer near Kristiansund, *H. Printz*, UC), Atlantic France in 1946 (Iles de Glénan and Concarneau, *R. Lami*, PC), and England apparently in 1939 (Yealm Estuary, at Steer Point, S. DEVON, *O. D. Hunt*, BM). The place of origin seems most likely Japan.

These two subspecies of *C. fragile* are easily distinguishable anatomically. The apices of the clavate-cylindrical utricles of subsp. *atlanticum* are rounded, extrorsely umbonate, or provided with a very short, usually blunt mucron. The utricles of subsp. *tomentosoides* tend to be shorter and stouter, have a pronounced median or submedian constriction, and are sharply mucronate.

Subsequent to Cotton's demonstration of *C. mucronatum* (*C. fragile*) in the British Isles, the name *C. tomentosum* remained to encompass the non-mucronate dichotomous forms. That two distinct species are involved has not been recognized heretofore.

Codium tomentosum and *C. vermilara* differ sufficiently in habit to be distinguishable in the field with a little practice. In *C. tomentosum* branching is usually regularly dichotomous and the branches are often flattened at the dichotomies; the base of the thallus is thick and terminal segments are often long. In *C. vermilara* branching is usually somewhat irregular, often proliferous, at times divaricate, and the branches are strictly terete; the base of the thallus is not noticeably thick and terminal segments are usually short. *C. tomentosum* is often more robust and more distantly branched than *C. vermilara*. Herbarium specimens are not so readily distinguishable on the basis of habit.

Anatomically the two species are unmistakable. The position of hair scars is significant: in *C. tomentosum* they are well below the apex and at a constriction, so that the utricle expands above them; in *C. vermilara* they are usually high on the shoulders of the utricle, or if lower, never at a constriction, so that the utricle tapers rather than expands immediately above them. The average length/breadth ratio is greater in *C. tomentosum* than in *C. vermilara*. Thickened apices are common in both species: in *C. tomentosum* a blunt point is frequently formed while in *C. vermilara* the thickening is more uniform. The cylindrical or narrowly clavate utricles of *C. tomentosum* are in contrast to the clavate or pyriform utricles common in *C. vermilara*.

C. vermilara is common throughout the Mediterranean (including the Adriatic). In the Atlantic it extends from Morocco to France and the British Isles. Aalesund and Kristiansund in Norway constitute the most northerly records. *C. tomentosum*, on the other hand, is primarily an Atlantic species, occurring from the British Isles south along France, Spain, and Portugal to Morocco. It occurs in the Mediterranean only along the Algerian coast. Heretofore, *C. tomentosum* has been credited with cosmopolitan distribution, but careful study reveals that more than thirty distinct species have been confused under this name.

Separating the two non-mucronate species in the British Isles was much easier than deciding their correct names. Application of the type method was made difficult by the absence of holotypes. The earliest name applicable to a dichotomous *Codium* would seem to be Hudson's *Spongia dichotoma* (1762, p. 489) or its isonym *Fucus tomentosus* (1778, p. 584). However, as Linda Newton (1953, p. 403) has explained, Hudson's binomials must be considered illegitimate synonyms of *F. elongatus* L. (the type of which is referable to *Himanthalia*), which Hudson cited as a synonym. This unfortunate circumstance resulted from the erroneous citation by Linnaeus of Morison's *Fucus spongiosus teres ramosior viridis erectis* (1699, p. 647, sect. 15, pl. 8, fig. 7), which misled Hudson to cite the Linnaean binomial. Miss Newton concluded, as I have concluded, that *Codium tomentosum* Stackhouse (1797) may be treated as a new name (rather than a transfer of *Fucus tomentosus* Hudson) in accordance with Art. 81 of the International Code of Botanical Nomenclature, inasmuch as Stackhouse explicitly excluded *Fucus elongatus* L. from his concept of *Codium tomentosum*.

In determining the application of the name *Codium tomentosum* Stackhouse, one is faced with the problem that Stackhouse had both non-mucronate species in hand, as shown by specimens in the Linnean Society. His description fits either species; the plant figured on Plate VII of his 'Nereis' is very likely *C. vermilara*. On the basis of the following considerations, however, it seems advisable to fix the application of *C. tomentosum* as delimited herein: (i) *C. tomentosum*, rather than *C. vermilara*, was the basis of treatments given by Turner (1811, p. 1, pl. 135), Harvey (1846, pl. XCIII), and Cotton (1912, p. 113, pl. VIII, figs. 1, 2); (ii) shifting the name *C. tomentosum* to *C. vermilara* would leave *C. tomentosum* as circumscribed herein without a name.

It is of historical interest to determine the identity of Morison's, Ray's and Hudson's plants. The two specimens in Herb. Morison (OXF), representing *Fucus spongiosus teres ramosior*...and *Fucus spongiosus teres viridis*...(1699

p. 647, nos. 7, 8), are both referable to *C. tomentosum*. The *Spongia dichotomos compressa, ex viridi splendens* (Ray, 1724, p. 29, no. 4) in Herb. Sherard (Oxf) is also representative of *C. tomentosum*. Hudson had both species in hand, judging from specimens at Kew, the British Museum, and Edinburgh.

Codium vermilara, occurring as it does in the Mediterranean, might be expected to have been described early. Although no authentic specimens have been located, the identity of Olivi's *Lamarckia vermilara* (1792), to judge from the description and figure, seems certain. While the lack of any reference to Olivi raises doubt that *Codium vermilara* Delle Chiaje (1829) should be considered a new combination, closer study of the rare Delle Chiaje work answers the question. It is clear from Delle Chiaje's choice of epithets in *Codium* that he was following Rafinesque (1810, p. 97), who in turn cited Olivi. Thus it seems proper to use the binomial *C. vermilara* (Olivi) Delle Chiaje.

Summary

The dichotomous species of *Codium* in the British Isles are described, compared, and illustrated. These include *C. fragile* subsp. *atlanticum*, *C. fragile* subsp. *tomentosoides*, *C. tomentosum* and *C. vermilara*.

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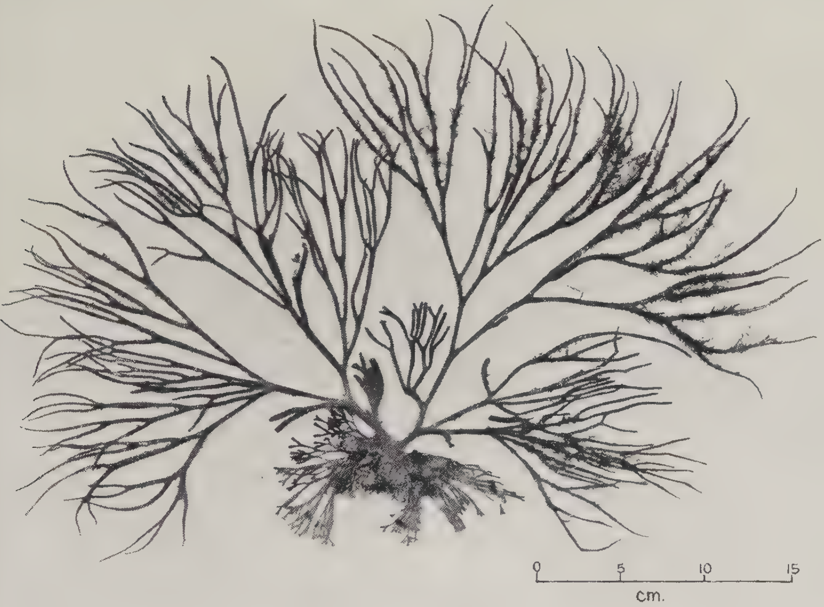

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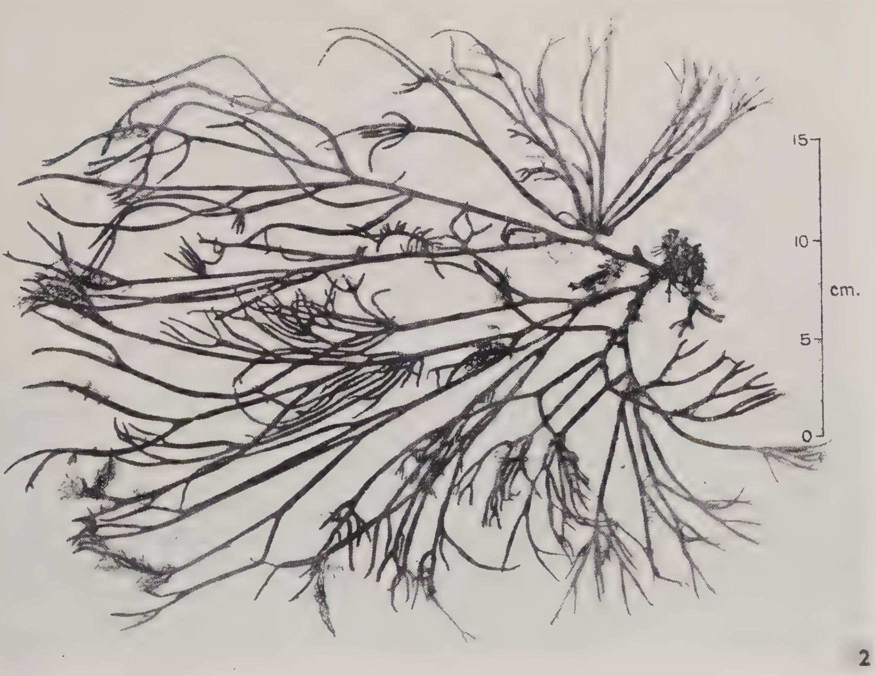

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EXPLANATION OF PLATE I

Fig. 1. *Codium tomentosum.* Small deep pool, Church Reef, Wembury, Devon, Oct. 1953, *F. G. C. Ryder* (UC).

Fig. 2. *Codium vermilara.* Large pool, mid-littoral, Church Reef, Wembury, Devon, Oct. 1953, *F. G. C. Ryder* (UC).

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STUDIES ON MARINE FLAGELLATES

II. THREE NEW SPECIES OF *CHRYSOCHROMULINA*

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(With total of 81 Figures in text and on Plates I–IX)

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INTRODUCTION

The three new species to be described below are the first of a considerable series of related forms, recently isolated from marine plankton off the British coasts and possessing in common a number of rather unusual characters which make their classification difficult. They are all very small and extremely fragile unicellular flagellates, with the characteristic golden brown plastid colour and metabolic products found in the Chrysophyceae to which they undoubtedly belong. Within this group their placing is more difficult and it cannot even be adequately discussed until the whole range of new forms is available for comparison. The most that we can do on this occasion is to make some provisional generic comparisons in order to establish a system of nomenclature. Unfortunately, existing descriptions of relevant genera are both scanty and incomplete, a defect which is easily explained by the nature of the organisms and the need for special facilities for their adequate study. The generic identification which we propose to adopt is therefore provisional to the extent that the little known genus *Chrysochromulina*, into which we believe our organisms can be placed, is founded on one freshwater species which is described in terms which will need substantial revision if this identification is to be accepted. Generic revision must, however, wait until the whole range of forms has been described, and we must therefore limit the present discussion to our reasons for the choice of the name.

These reasons are essentially that our forms and *Chrysochromulina* agree in possessing three rather long filiform appendages, two of which are flagella. In the only other Chrysophycean genera for which three appendages have been recorded, namely *Prymnesium* and *Platychrysis*, the third appendage is so short as scarcely to justify the epithet filiform, and it differs in other ways (to be discussed on a later occasion) which do not suggest identity. The third appendage in the only species of *Chrysochromulina* (*C. parva* Lackey), on the other hand, was described as a third flagellum (Lackey, 1939). This attribution is not borne out either by the description of its behaviour or by the figure which accompanies the description. Both resemble those of our organism sufficiently to make it virtually certain that the third organ in *C. parva*, as in our organism, is not a flagellum but an organ of very different properties and use.

In our organisms the third appendage is a retractile organ with an adhesive tip, in some species (though not in those to be described here) extensible to many times the length of the flagella and in all used for temporary anchorage of the cell to a solid surface, though this may perhaps not be its sole function. This power of anchorage is apparently correlated (though in ways which are not yet fully understood) with the practice of phagotrophic feeding, floating particles of various sizes being engulfed through the end of the cell away from the attachment of the appendages. This notwithstanding, the organisms are capable of photosynthesis and under normal conditions require light; their dependence on solid food for certain nutrients, however, explains the difficulty encountered in making unialgal cultures bacteria-free.

In addition to the various characteristics associated with the third appendage, all our species show a covering of scales when seen under the electron microscope; such scales being apparently of an organic nature since we can find no sign of mineral impregnation either calcareous or siliceous. They are of very varied sizes and shapes and in themselves they would provide most excellent diagnostic characters not only for the delimitation of species but also in all probability for the separation of several genera each with several species in our range of material alone. Unfortunately their small size, which makes the scales invisible with the light microscope in almost every species, would lead to great difficulties of identification of such genera by the ordinary student of plankton. While therefore we cannot avoid mentioning such characters—even if this introduces some doubt about specific identifications by a light microscopist—we do not propose to found genera on characters which cannot be seen with the light microscope. We therefore propose to describe most of our species under the one genus *Chrysochromulina*, and to amend the generic diagnosis to incorporate the new knowledge at the end of the series.

Since, however, it is obvious that an essential part of any generic diagnosis must involve a description of the third filiform appendage formerly referred to as a third flagellum, it will be convenient if we give it at once some name less misleading than flagellum and less cumbersome than 'third filiform appendage'. We therefore propose to call it a *haptonema* (Gr. ἅπτω, to attach, and νῆμα, pl. νῆματα, a thread) to give it a term of its own without reference to other morphological categories. The full description of its structure and properties will then emerge as the species are themselves described and further investigated.

MATERIALS AND METHODS

The establishment of unialgal cultures has been an essential pre-requisite to the effective study of the structure and behaviour of these organisms. The methods of isolation have been in no way peculiar and the cultures so established have been successfully maintained for several years at Plymouth in Erdschreiber culture solution in a culture room kept at about 15° C. and illuminated with fluorescent lighting to supplement limited daylight from a north window.

For studying the behaviour of the living cells, especially the motion of their various appendages, the use of the modified dark ground condenser of Cooke, Troughton and Simms, illuminated by a Pointolite lamp, has been of the very greatest help. Unfortunately photography at high magnifications is not possible with this system without a more powerful source of light than has been available to us. It is, however, the best means of demonstrating the haptonema to a visual observer.

Without dark ground it is possible, if the cells are not moving rapidly, to see the appendages quite well under an oil immersion objective or a good dry lens without special lighting, but recording them photographically by this means is extremely difficult (see, however, Fig. 24, Pl. I; Fig. 55, Pl. V; and Fig. 77, Pl. VIII). They can also be picked out by phase-contrast or ultraviolet photography, but in the smallest organisms such as those to be described here the optical interference caused by the body may be a serious difficulty. It was therefore found by experiment that the best way of recording these structures is to dry the cells down on to a glass slide after preliminary fixation with either osmic vapour or other simple killing agent such as iodine in KI. It is sometimes an advantage to precede killing by narcotizing with magnesium chloride to prevent retraction of the haptonema. After the preparation has been dried, it must be freed from the salt crystals by immersion in distilled water, followed by a second drying. This part of the process does not appear to disturb the attached cells which, after removal of the salt which otherwise will deliquesce, can be kept indefinitely in the dry condition. The appendages can then be seen even with a relatively low-power dry lens and for photographic purposes the fact that they are spread on a flat surface is a great advantage. They must, however, be examined dry, and so the use of high resolution lenses is precluded.

Formalin fixation, as commonly used for plankton studies, is quite inappropriate for these organisms. The appendages are invariably lost, the cell bodies become bloated, collapsed or otherwise misshapen and the chromatophores are indistinguishable. The organisms are then unrecognizable.

For investigating the feeding habits, and in particular for demonstrating the ingestion of solid food, it is sufficient to supply the cultures with suitable coloured particles and to leave them overnight undisturbed. By next morning the ingested particles can be detected inside the cells and recorded photographically. Various pigments such as carmine, or cultures of small coloured micro-organisms, have been used for this purpose successfully, but the most satisfactory both for its apparent palatability to almost all the organisms and also for purposes of photographic record have been the densely black particles of fine graphite sold under the name of 'Aquadag'. It is desirable to wash this several times in culture fluid before use to avoid risk of chemical contamination, after which it is readily ingested and very clearly visible.

The application of intravitam stains, notably cresyl blue, is a help for demonstrating the presence, though not the details, of the surface covering of scales and of the ejectile organelles which will be mentioned in the descriptions. The scales appear as a pale rose-violet line which could not be distinguished optically from a simple membrane.

For the electron microscopy the methods used have been those previously applied to other types of motile cells (see Manton & Clarke, 1951, *et seq.*) with the sole modification that the present material is considerably more sensitive than is usual and fixation times have to be shorter. It is sufficient to expose the live cells to the vapour from 2% osmium tetroxide solution for 5 sec to cause death and this is sufficient treatment for direct preparations killed on a formvar-coated carrier. After killing they are dried, and eventually shadowcast with gold-palladium.

Direct preparations of this kind are usually to be preferred for revealing the details of scales, but for the general form of the cell with its various appendages suitably spread it is often better to strip from glass as described in Manton, Clarke, Greenwood & Flint, 1952. This procedure makes possible a more extensive examination of alternative specimens before selecting a favourable cell for detailed study. Each of these methods will be represented in the plates.

In assembling our observations for publication we have attempted to obtain pictorial demonstration of all features which lend themselves to this treatment using photography where possible. For each species we have included photographs and drawings at a standard magnification with the light microscope, since such views are indispensable for the ordinary purposes of identification. For the relative lengths of the appendages with respect to the body size some verification is possible on the photographs, more especially of undried material, though the figures quoted were actually obtained from living material and from cells recently killed with osmic vapour, in each species more than 100 individuals having been measured. For the type of detail which can only be seen with the electron microscope some adjustment of magnifications is necessary in relation to the actual size of the organisms in order to fit the micrographs on to a limited page area. This may obscure the relative differences of size which exist between the species, though these can at once be apprehended from the visual and low-power views assembled on the first plate of each species. It should also perhaps be pointed out that at high magnifications the performance of the electron microscope is liable to slight fluctuations and the values given on the remaining plates are therefore only approximate.

The electron microscope used has been the Philips instrument in the Leeds University Botany Department, except for Fig. 61, Pl. VI, which was taken on a similar instrument in the research laboratory of Messrs Tootal Broadhurst Lee Co. Ltd of Manchester, whom we wish to thank for giving one of us temporary facilities for working there as a guest.

In the descriptive part of the text, for convenience, the detailed account of each species is preceded by a formal diagnosis in English and Latin. The subsequent descriptions can then be abbreviated to comparative notes to incorporate the Figure and Plate references. The first species will be described the most fully and reference to it must be made for certain details common to all three species.

We wish to thank the following for special help: Dr J. E. Morton of Queen Mary College for translating the English diagnoses into Latin, Dr L. H. N. Cooper of the Plymouth Laboratory and Messrs D. Vaux and A. C. Burd of the Ministry of Agriculture and Fisheries Laboratory, Lowestoft, for bringing in the samples of sea water from which these organisms have been isolated, and Miss D. Ballantine for testing their possible toxicity to fish.

SPECIFIC DESCRIPTIONS

Chrysochromulina kappa n.sp., Parke & Manton

Diagnosis

Motile cells sphaeroidal, showing some metaboly, 4·5–6·5 (exceptionally 4–10·5) μ in diameter. Two flagella and one haptonema[1] arising close together at one pole; the flagella equal, homodynamic, 1½–2½ times the cell diameter in length, smooth, shortly mucronate at the tip under the electron microscope; the haptonema a little longer than the flagella when fully extended, with a basal swelling, a club-shaped tip and a translucent sheath visible only with the electron microscope. The periplast covered by very thin transparent sculptured scales visible only with the electron microscope, on any one cell ranging in shape from oval to polygonal and in size from $0{\cdot}3\times0{\cdot}4\,\mu$ to $0{\cdot}5\times0{\cdot}8\,\mu$ with, at the flagellar pole, a few scales bearing a soft central spine.

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[1] For derivation and use of this term see p. 581.

Cells uninucleate, no stigma. Chromatophores usually 2 or 4, sometimes 1 or none, golden brown; in cells of the motile phase usually saucer-shaped, parietal, with a single globular body (pyrenoid?) placed centrally on the internal face of each; in cells of the non-motile phase deeply lobed or stellate. Oil and leucosin produced. Ejectile muciferous bodies localized in the cytoplasm mainly at the non-flagellar pole. Nutrition holophytic and/or phagotrophic. Not toxic to fish.

In the motile phase asexual reproduction of pigmented and non-pigmented cells by fission into 2, 3 or 4 daughter-cells. In the non-motile phase reproduction (asexual?) by successive fission of amoeboid cells to produce 4 daughter-cells with walls; motile phase probably liberated from walled daughter-cells through a pore.

Habitat: the sea off Port Erin, Isle of Man (15 June 1939, type culture) and off the south coast of England at position Lat. N. 49° 51′, Long. W. 04° 00′ (8 April 1950) at 10 m. and Lat. N. 49° 21′, Long. W. 04° 54′ (9 May 1950) at surface. Type culture (Plymouth collection 'K') deposited with the Type Culture Collection, Cambridge, and the Marine Biological Association; preserved material and photographs lodged with the Marine Biological Association, Plymouth, England.

Cellula motili sphaeroidali, paululum formam mutanti, $4{\cdot}5\,\mu$ ad $6{\cdot}5\,\mu$ (rare 4 ad $10{\cdot}5\,\mu$) latitudine; duobus flagellis et unico haptonemate (vide p. 581) conjunctim exorientibus altero axi; flagellis similibus, homodynamicis, longioribus $1\frac{1}{2}$ ad $2\frac{1}{2}$

Legends to Text-figs. 1–12

Chrysochromulina kappa n.sp. (×5000)

Fig. 1. Individual swimming with flagella and haptonema behind body in the position characteristic for the species during rapid swimming. *c*, chromatophore; *f*, flagellum; *h*, haptonema; *l*, leucosin vesicle; *m*, muciferous body; *n*, nucleus; *p*, pyrenoid-like body; *s*, scales; *ss*, spined scales.

Fig. 2. Individual anchored by haptonema which is scarcely extended; cell containing recently ingested graphite particles (*g*).

Fig. 3. Anchored cell with haptonema partly extended; cell ejecting large mass of graphite.

Fig. 4. Swimming cell containing large mass of graphite.

Fig. 5. Individual swimming with flagella and haptonema in front of body, haptonema fully extended; graphite particles being ingested.

Fig. 6. Swimming individual lacking chromatophores; an ingested bacterium at non-flagellar pole.

Fig. 7. Early fission stage: 4 flagella, 1 haptonema, 4 pyrenoid-like bodies, chromatophores dividing.

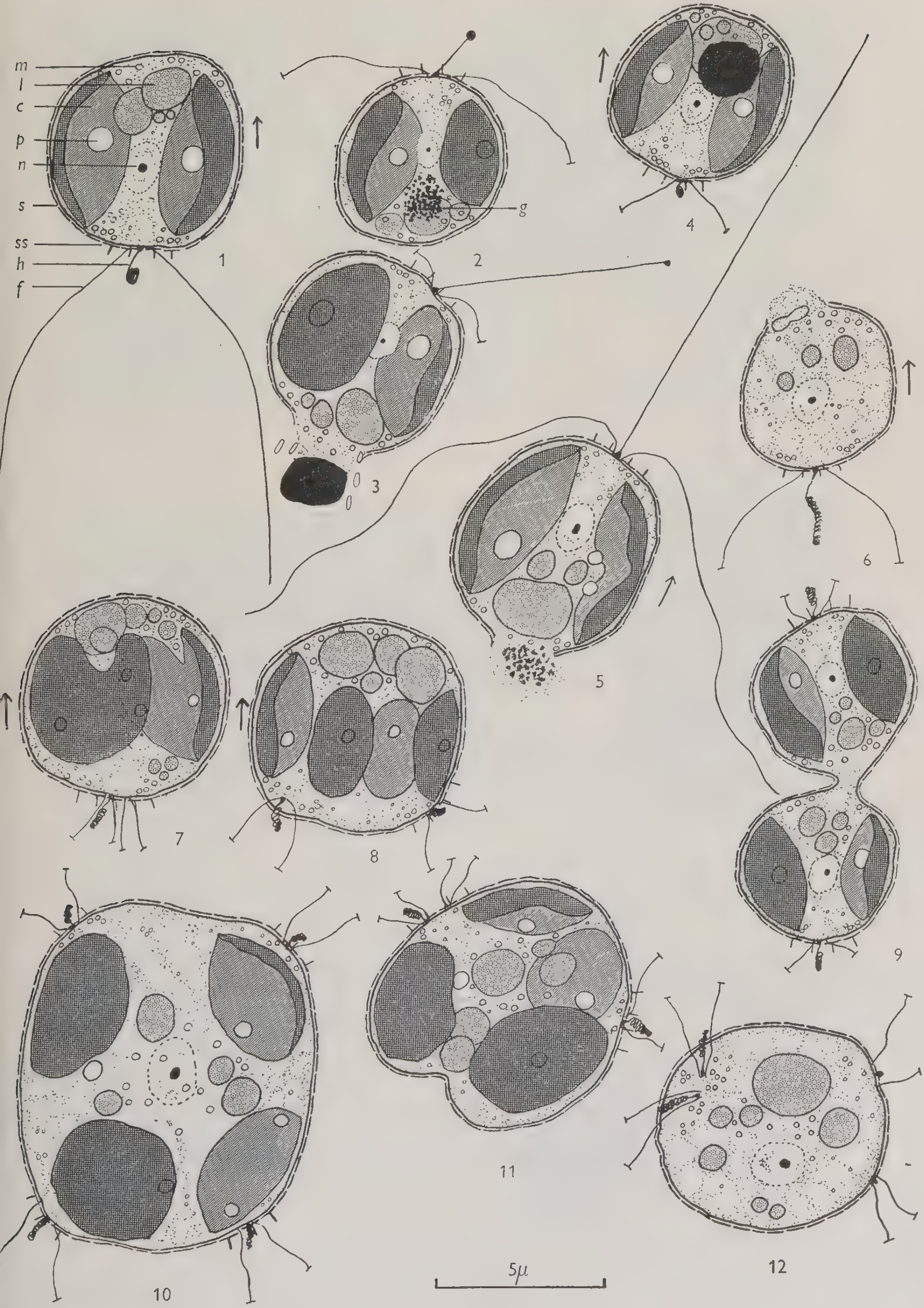
Fig. 8. Fission stage: flagella separating, 2nd haptonema formed, 4 chromatophores.

Fig. 9. Late fission stage just before separation of daughter-cells.

Fig. 10. Stage with 4 pairs of flagella and 4 haptonemata before double-fission to give 4 daughter-cells.

Fig. 11. Early fission stage to give 3 daughter-cells.

Fig. 12. Colourless individual with 4 pairs of flagella and 4 haptonemata prior to double-fission.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Text-figs. 1–12.

cellulae latitudine, teretibus, breve mucronatis ut videantur per microscopiam electronicam; haptonemate, cum maxime extensus sit, paululum longiore quam flagellum, basaliter leve tumescenti, ad extremitatem clavuloso, induto pelliculo diaphano visibili solum per microscopiam electronicam. Periplasto induto delicatissimis squamis diaphanis et sculptis, visibilibus solum per microscopiam electronicam; squamis figura ovalibus aut polygonalibus, 0·3 ad 0·5 μ lat., 0·4 ad 0·8 μ long.; nonnullis squamis ad situm flagelli exoriendi ornatis unaecuique molli spinulo centrali.

Nucleo unico, stigmate nullo; chromatophoris ex norma 2 aut 4, nonnunquam unicis aut nullis, aureo-fulvis; in cellulis status motilis chromatophoris ex norma crateriformibis, parietalibus, unocuique unico largi corpore globulari (pyrenoidali?) posito ad centrum aspectus concavi; in cellulis status non-motilis chromatophoris profunde lobatis vel stellatis. Cellulis oleum leucosinumque parientibus. Corporibus ejectilibus muciferosis locatis praecipue ad regionem cytoplasmatis objectam axi flagellari. Nutritione holophytica et tamen phagotrophica. Non toxica piscibus.

Generanti asexualiter in status motili per fissionem cellularum coloratarum vel incoloratarum in 2, 3 aut 4 cellulas filiolas. In statu non-motili generanti (asexualiter ?) per fissionem, aliae post alias, cellularum amoeboidalium ad 4 cellulas filiolas cum parietibus. Potest ut cellulae motilis status per foramen ex cellulas filiolas tiberentur.

Habitat mare prope Port Erin, Isle of Man (15 Jun. 1939, cultura typica).

Description

The sphaeroidal shape of the motile cells is demonstrated in the drawings of Figs. 1–12 and in the photographs of Pl. I, especially Figs. 22, and 24–26 which refer to undried specimens not deformed by shrinkage. The slight metaboly, particularly at the non-flagellar pole, can only be demonstrated on living material. In an actively growing culture 65% of the cells are from 5 to 6·5 μ in diameter, while 17% are between 4·0 and 5·0 μ. The remaining 18% are incipient division stages (including double-fission stages) and range from 6·5 to 10·5 μ in size.

The origin of the three appendages near together at one pole of the cell is clearly shown in the three electron micrographs assembled on Pl. II. These also show the bulbous base to the haptonema and its club-shaped tip. The sheath is best seen in Pl. III. The relative lengths of the three appendages

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Legends to Text-figs. 13–19

Chrysochromulina kappa n.sp. (× 5000)

Fig. 13. Two amoeboid individuals showing slightly lobed chromatophores.

Fig. 14. Large amoeboid individual with 4 deeply lobed, pale chromatophores.

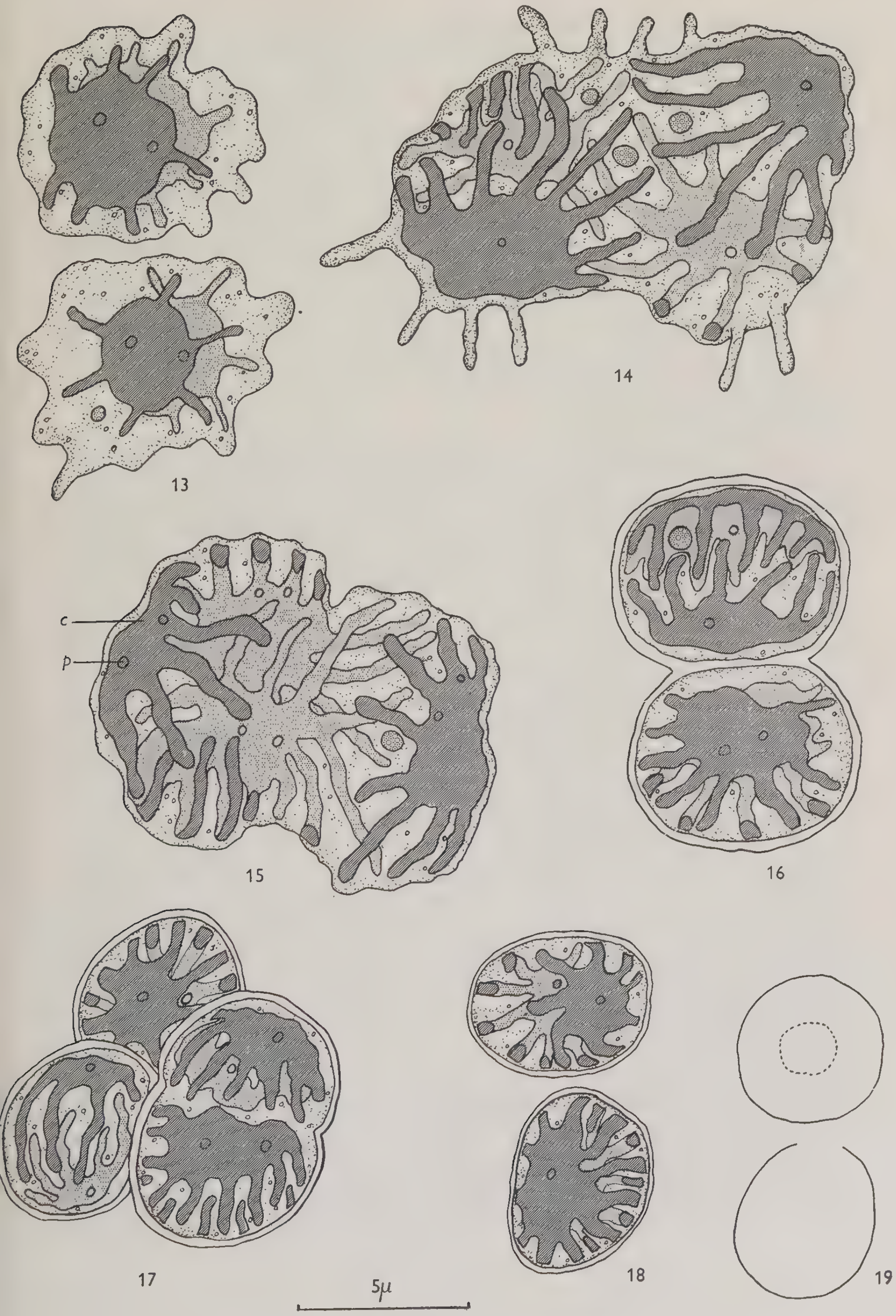
Fig. 15. Early fission (?) stage of a large individual: 4 chromatophores (*c*), 8 pyrenoid-like bodies (*p*).

Fig. 16. First fission of a large, walled cell just completed.

Fig. 17. Second fission of a large, walled cell almost completed to give 4, small, walled daughter-cells.

Fig. 18. Small, walled daughter-cells, separated, each with two stellate or deeply lobed chromatophores.

Fig. 19. Empty walls of small daughter-cells showing pore through which motile stage has probably been liberated.


13
14
c
p
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16
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5μ

Text-figs. 13–19.

compared with the body size needs to be ascertained on specimens which have not been dried, to avoid falsification by body shrinkage. Figs. 22 and 24 on Pl. I are suitable for this purpose.

Details of the body scales can be seen at a low magnification in Pl. II and at higher magnifications in Pl. IV. Characteristic of them is their extreme transparency to the electron beam in which they contrast markedly with the silicified scales of a form like *Synura* (Manton, 1955) or with calcified coccoliths (Braarud *et. al.*, 1952; Braarud, 1954), which are intensely opaque to electrons, appearing black in the electron microscope. Our failure to detect any chemical evidence for mineral impregnation of the scales in any species of *Chrysochromulina* so far tested is no doubt correlated with these optical properties, and since the scale covering stains with cresyl blue sufficiently strongly for it to be detected by the light microscope as a distinct rose-violet line outside the periplast, there must clearly be an organic basis to the scales which may in fact be their sole component.

The general shape of the scales is plate-like with a delicate surface sculpturing of radiating lines which become enhanced by shadow-casting and which may therefore best be examined in the reversed prints of Figs. 34 and 35, Pl. IV (compare with Fig. 33). In the smallest oval scales (Fig. 35) these ridges extend to the extreme edge of the scale producing a crenate margin; the larger polygonal scales (Fig. 34) possess a raised rim which obscures the ends of the ridges. Occasionally, as on the left of Fig. 34, polygonal rims can be found surrounding little or no traces of a central disk; it is not, however, known whether these are perhaps immature scales or whether the rim is in fact detachable from the disk which in such cases may have fallen out. Since the scales can only be studied after they have become detached from the body we have no means of determining the precise details of distribution on the body of the various sizes and shapes. The relatively few spined scales (Fig. 35) are, however, so conspicuously grouped near the flagellar pole (cf. Fig. 33, Pl. IV) that we think that they must be limited to this region. As may best be seen in Fig. 35, the heads of such scales are polygonal with a raised rim and faint radiating lines, though the four coarse ridges subtending the spine (which is usually collapsed after drying in this material) are possibly not entirely preformed structures but could partly be folds. They are invariably present in dried material but the details of their configuration are less constant than are those of the pattern of true surface sculpturing. The biological significance of these scales and the details of their modes of production are wholly unknown.

Since the cell body is too large to be penetrated by the electron beam in whole mounts, our observations on the internal cell organs are at present confined to the light microscope. The saucer-shaped parietal chromatophores of the motile phase are shown in the drawings of Figs. 1–5, and 7–11, with the stellate chromatophores of the non-motile phase in Figs. 13–18. Sometimes the chromatophores of the motile stage appear striated, their number is usually two and we have never found more than four, the latter being in most cases early division stages. Cells with one chromatophore and division stages with three chromatophores are occasionally seen. Individuals which lack chromatophores are also occasionally seen, though they are always of small size, 4–4·5 μ in diameter (Fig. 6).

A conspicuous refringent body, perhaps equivalent to the so-called pyrenoids of some other authors (see below), is situated near the centre of the inner face of each chromatophore (Figs. 1–5, 7–11). These bodies vary in size and sometimes appear to be surrounded by non-refringent material. They appear to divide at the same time as the chromatophores (Figs. 5, 7), but it is not known whether they are actually attached to the inner face of the chromatophore since they have sometimes been observed apparently lying away from it. They do not stain with acid fuchsin, iodine, neutral red or cresyl blue, but with Millon's reagent they become gold, pink or rose-red. The 'pyrenoids' of certain naviculoid diatoms were found to become orange-gold after treatment with Millon's reagent (Tschermak-Woess, 1953), and those of the coccolithophorid *Pontosphaera roscoffensis* showed a comparable negative reaction with other common reagents (Chadefaud & Feldmann, 1949). After osmic fixation these bodies in *Chrysochromulina kappa* become very conspicuous, sometimes appearing a vivid green. A similar behaviour has been found (Dr J. W. G. Lund personal communication) in the 'pyrenoids' of the chrysophycean *Apistonema pyrenigerum*.

The nucleus is sometimes visible in the living cells; it is of medium size and is placed centrally in the body, usually slightly nearer to the flagellar pole (Fig. 1). Vesicles of leucosin, varying greatly in number and size in different individuals, are present, usually towards the non-flagellar pole, but occasionally small leucosin vesicles are also present near the flagellar pole (Fig. 7). Small oil globules are distributed throughout the cytoplasm.

Very small refringent organelles, possibly corresponding to the muciferous bodies of some workers (Chadefaud, 1936), can be seen in the peripheral cytoplasm. No great number are present in cells of this species (Figs. 1–12), and they seem to be localized mainly near the non-flagellar pole, but small groups are also present round the flagellar pole (Figs. 1–6). Under bright illumination, or when very dilute cresyl blue is added to the live cells, these organelles shoot out their contents as small rods or batons if extruded quickly (Fig. 3) or, alternatively, their contents may ooze out as small balloons which can enlarge to equal the cell in diameter. Immediately before the rods are shot out, very small disks appear to be shot off in a manner reminiscent of the behaviour described by Hovasse (1949) for the discobolocysts of *Cyclonexis annularis*. In *Chrysochromulina*, however, it seems probable that these disks are surface scales which are removed as the organelles discharge their contents. The biological significance of these discharges is unknown.

Explanation of Plates I–IV

Chrysochromulina kappa n.sp.

I

Fig. 20. A cell killed with osmic vapour and dried on a glass slide. Photographed dry, without a coverslip. Magnification × 1000.

Fig. 21. Another cell of the same.

Fig. 22. A cell killed with osmic vapour and photographed in liquid culture medium under a sealed coverslip; the two flagella only but not the haptonema visible in the plane of focus chosen. Visual light, oil-immersion objective, magnification × 1000.

Fig. 23. A cell treated as for Fig. 20 but killed and dried on quartz before mounting in water with a trace of iodine in KI under a sealed quartz coverslip. High power U.V. photograph, glycerine-immersion lens, magnification × 3000.

Fig. 24. Parts of two undried cells from the preparation of Fig. 22 photographed with visual light 24 h after the preparation was set up. The protoplasm has darkened slightly in the dilute osmic solution resulting from vapour killing. The coiled haptonema and two flagella are visible for both cells though the body of the left-hand cell has broken away. Visual light, oil-immersion objective, magnification × 2000.

Fig. 25. An undried cell showing the effects of graphite feeding, otherwise like Fig. 22. A pellet of ingested graphite is in focus between the two plastids at the pole away from the flagella. Visual light, oil-immersion objective, magnification × 2000.

Fig. 26. Another cell of the same showing a larger pellet of ingested graphite.

Fig. 27. Low-power electron micrograph of a cell killed with osmic vapour and dried directly on the formvar film. Electron micrograph M. 113, 27 gold palladium shadowing, 60 kV, magnification × 3000.

II

Fig. 28. A cell killed with osmic vapour directly on the formvar film. The body, scales, coiled haptonema and the two flagella, are visible, with a bacterium and a *Caulobacter* type of organism touching the tip of the right-hand flagellum. Electron micrograph M 120·26, 60 kV, magnification × *c.* 6000.

Fig. 29. Another cell of the same showing the uncoiled haptonema and its bulbous base. Electron micrograph M 121·1, 60 kV, magnification × *c.* 6000.

Fig. 30. Parts of two other cells of the same: the right-hand cell showing the tightly coiled haptonema breaking away just above its bulbous base, the left-hand cell disrupted, the bulbous base and the two flagella alone remaining. Electron micrograph M. 122·14, 40 kV, magnification × *c.* 6000.

III

Fig. 31. Part of a cell similar in treatment to those on Pl. II, showing the coiled haptonema with its sheath. (For a better view of the scales see Pl. IV.) Electron micrograph M 114·6, 60 kV, magnification × 20,000.

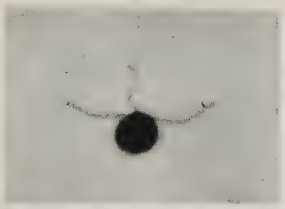
Fig. 32. Part of a similar cell with the haptonema partially extended, and fibrillar components visible in the flagella. Electron micrograph M. 114·4, 60 kV, magnification × 20,000.

IV

Fig. 33. Part of the right-hand cell of Pl. II, Fig. 30, to show the bulbous base of the haptonema and body scales more clearly. Electron micrograph M. 122·18, 40 kV, magnification × 20,000.

Fig. 34. Reversed print of part of the preceding to show sculpturing of scales more clearly Electron micrograph M. 122·18, magnification × 30,000.

Fig. 35. Similar details from another cell to show some small rimless scales and a collapsed spined scale, from the region of the flagellar bases. Electron micrograph M.182·19, 40 kV, magnification × 30,000.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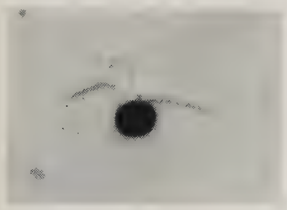
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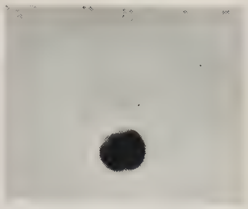
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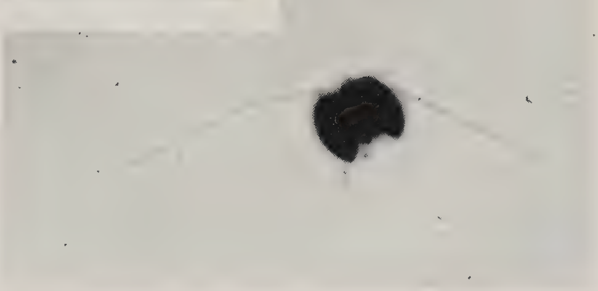
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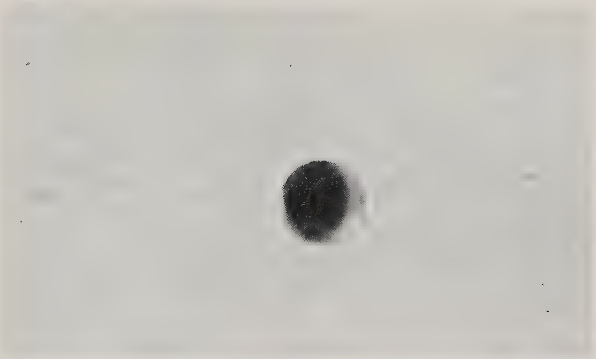
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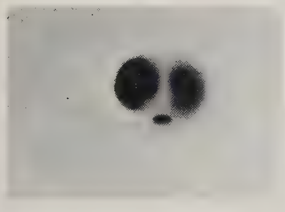
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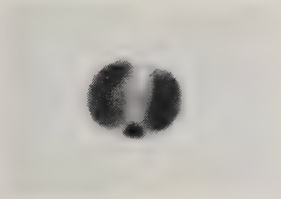
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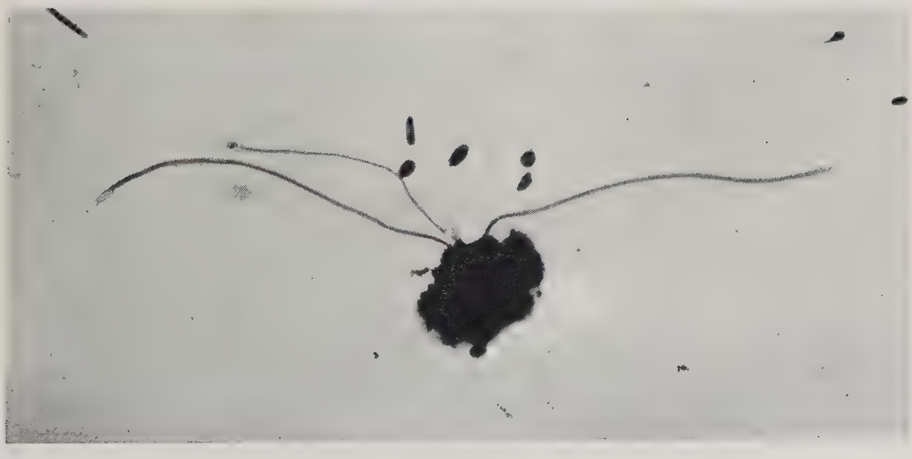
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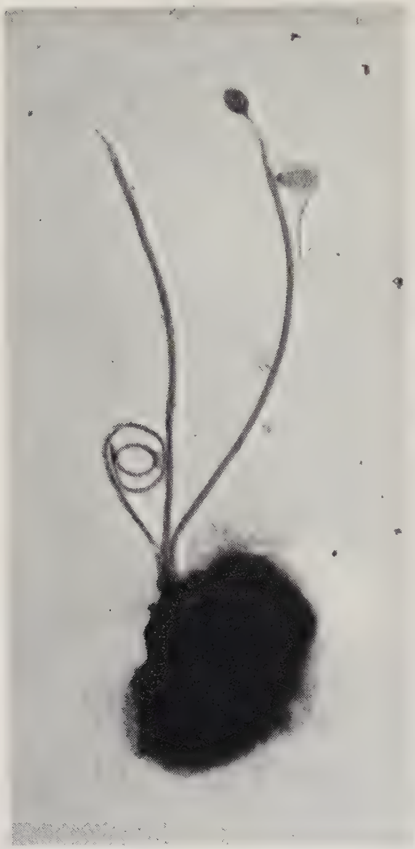
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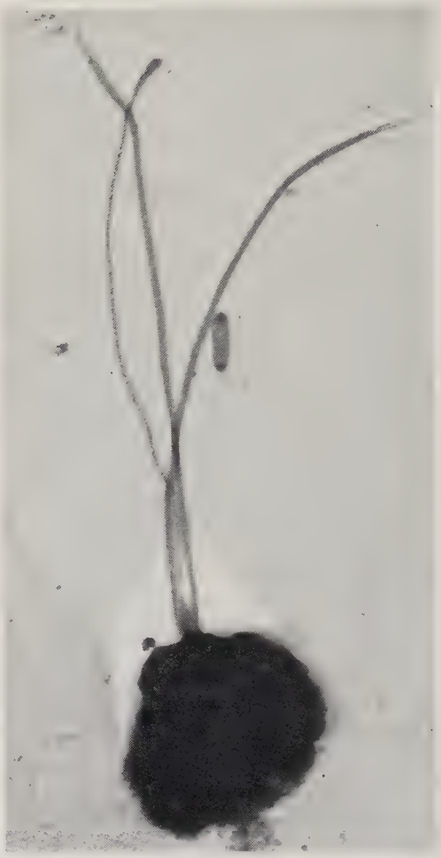
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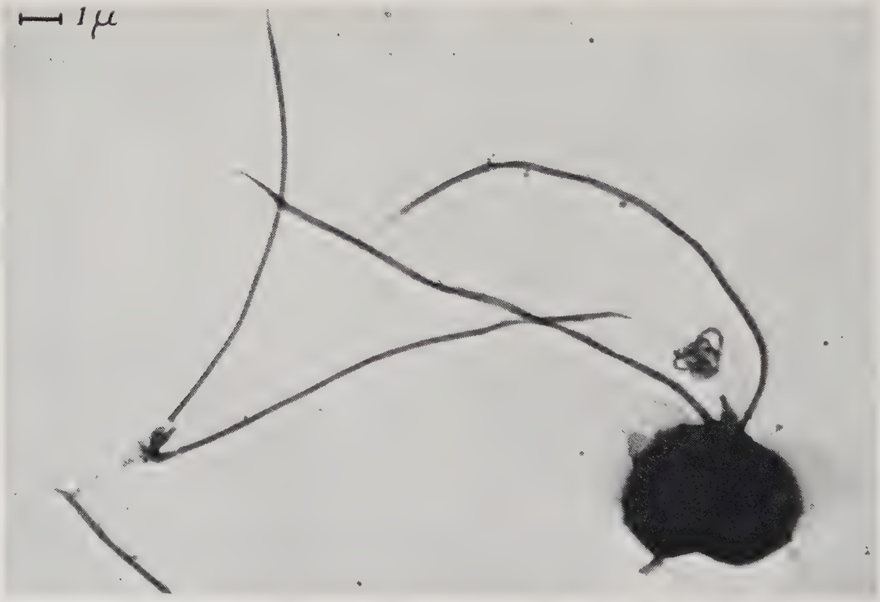

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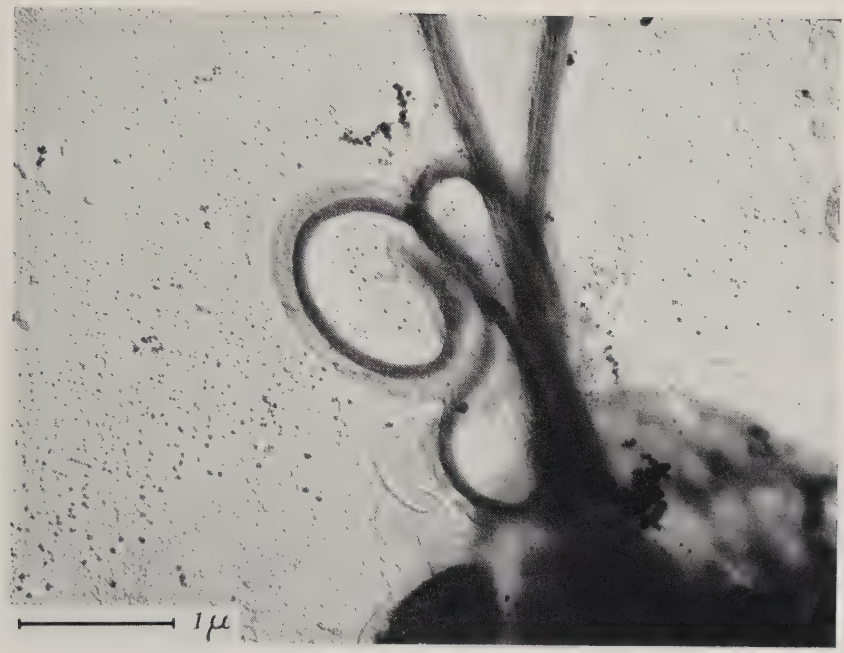

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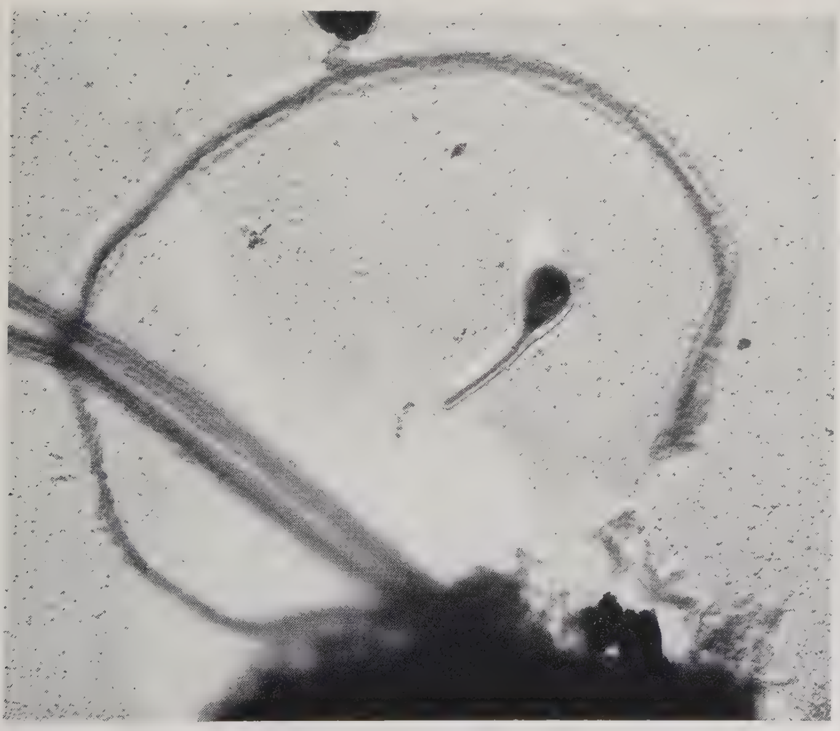

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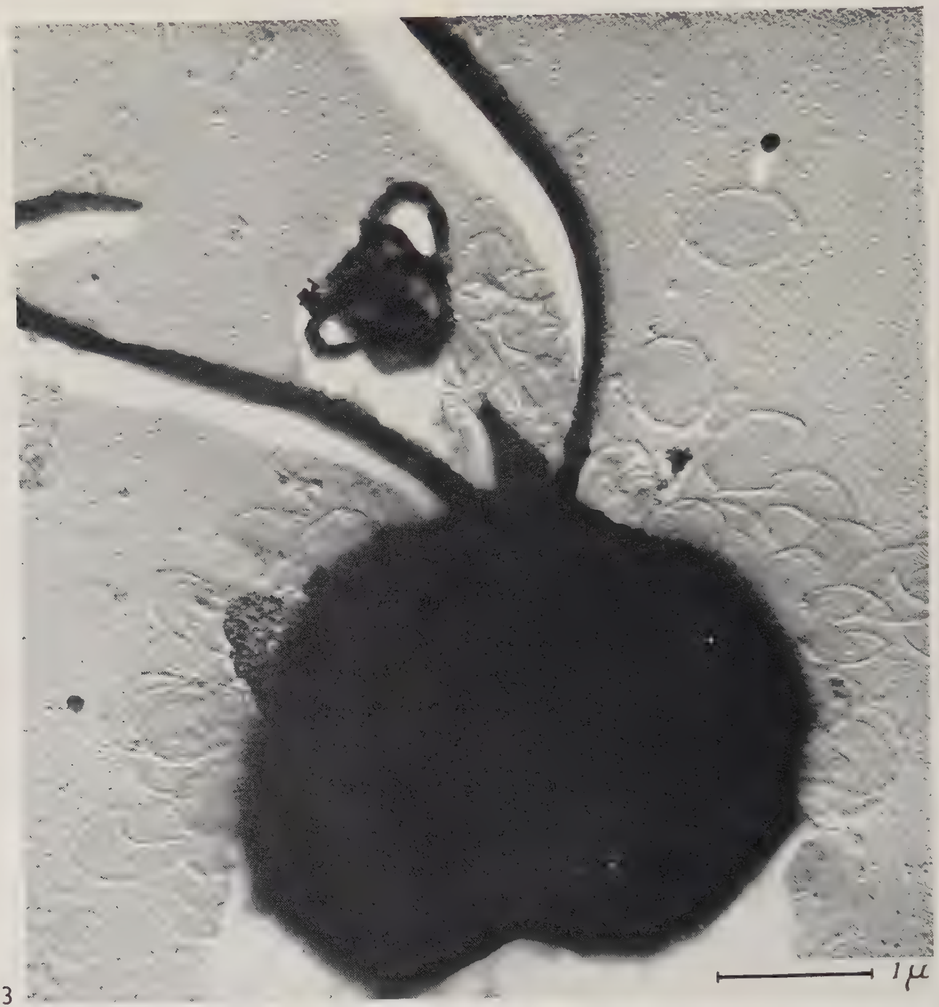

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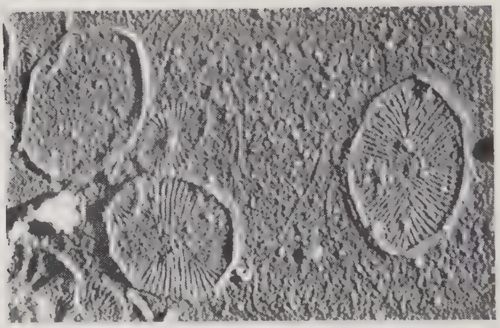

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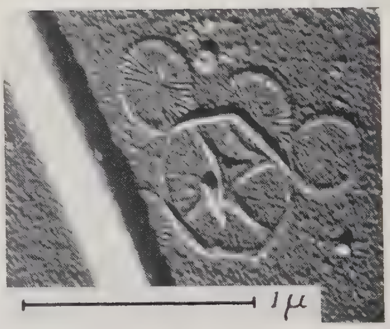

3

In the localized peripheral areas in which the muciferous organelles occur, a few granules stain bright blue with cresyl blue and are perhaps mitochondria. Two or three slightly larger masses stain a blue-green with this reagent; these are perhaps physodes (cf. Bourrelly & Magne, 1953; Magne, 1954).

The locomotory movements, which are some of the most significant attributes of these organisms, are unfortunately unsuited to diagrammatic demonstration, and drawings of a few characteristic attitudes to accompany a brief verbal description must suffice. In spite of the absence of a stigma, the motile cells show a marked phototactic reaction when exposed to uneven illumination in a Petri dish, accumulating preferentially towards, or away from, the light according to the intensity. When mounted under the microscope the cells move rapidly in rather large circles for a limited period, after which they slow down and either attach themselves temporarily to the surface of the slide or else move off again in a different direction. The cell body rotates and gyrates during swimming. When viewed under dark ground illumination the appendages are held as in Fig. 1 during periods of most rapid swimming, that is directed *backwards* with the haptonema almost or completely coiled up and the two vibrating flagella diverging at a wide angle so that their free ends are further apart than the width of the cell body. In this position the haptonema has never been seen fully uncoiled, and it is generally coiled so closely to the body that it may be difficult to see. When slightly uncoiled at the base (Fig. 6) the cells appears to move more slowly. Sometimes, however, individuals are seen moving with the flagella and haptonema directed *forwards*, when the haptonema is commonly extended to its full length (slightly longer than the flagella) and is held stiffly out in front of the body while the flagella splay out laterally (Fig. 5) showing an undulating movement.

At any moment after a period of active swimming the cells may slow down and anchor themselves by the tip of the haptonema to any sufficiently firm surface which under the conditions of viewing is commonly the surface of the glass slide. The flagella may continue to vibrate with undiminished vigour and the cell body then carries out peculiar shaking or dancing movements, or it may occasionally move slowly in a circle round the point of anchorage. These movements are characteristic of most of the forms examined by us with short haptonemata and they are an extremely useful character for quickly recognizing these organisms in a mixed sample. When attached by its distal extremity the haptonema can be fully extended, but usually it is partly or even almost wholly coiled up except at the base (Figs. 2, 3). When tightly coiled it is liable to be hidden beneath the body, but the characteristic movements will show that it is attached. When only a short amount is uncoiled at the basal end the remainder is coiled in a flat spiral appearing to the eye as a disk with the point of attachment in the centre. This disk-like appearance, however, can only be seen under an oil-immersion objective without which such an individual could momentarily be mistaken for a species of *Prymnesium*.

Phagotrophy is of common occurrence (Figs. 2–6), suspended particles, bacteria and small plant forms up to 2·5μ in diameter being ingested. The actual process of ingestment is difficult to analyse since the act itself is very rapid. Recently ingested cells of other organisms can sometimes be seen in Brownian movement within a vacuole. By the use of graphite it has been possible to ascertain with certainty that the site of ingestion is at the non-flagellar pole of the cell. A colourless, slightly granular, substance seems to flow out from this region to surround the particles (or cells) being ingested (Figs. 5, 6), which are then quickly drawn within the body (Fig. 2). Ingested particles at first seem to be moved about within the body but they are gradually compacted together, apparently within a vacuole, until they form a rounded pellet (Fig. 4; and Figs. 25 and 26, Pl. I). Material can be ingested, moved about in the cell, rounded off and then thrown out again (Fig. 3) all in the space of 5 min. In a very short time after graphite has been added to a culture individuals can be seen swimming quite normally with large masses of graphite inside them (Fig. 4). A cell 5μ in diameter is able to accommodate a mass as large as 2·5 × 2μ. This species tends to keep ingested material in the body fairly close to the pole at which it entered. When the graphite masses are ejected from the body some of the muciferous organelles (see p. 589) are commonly seen to discharge their contents as rods at the same time (Fig. 3). It is probable that all these processes can take place both when the cell is attached and when it is swimming.

Reproduction of the motile stage is by fission to produce usually two equal daughter-cells, each 4–5μ in diameter, but fission can give daughter-cells of unequal size. When fission is unequal the smaller daughter-cell occasionally lacks chromatophores. The two new flagella generally develop as the chromatophores are dividing (Fig. 7). The second haptonema then appears as the cell broadens to separate the two pairs of flagella prior to the actual fission (Fig. 8). But the chromatophores can divide, giving four in an individual, before the new flagella are produced, or the cell can divide without concurrent chromatophore division, in which case one chromatophore passes to each daughter-cell or one has two and the other none.

Occasionally in this species large motile cells, 9–10·5 × 7–9μ, are found (Fig. 10) which bear four pairs of flagella and four haptonemata and contain four chromatophores. These cells divide into four daughter-cells, each of which usually contains one chromatophore but occasionally one of the daughter-cells may contain two chromatophores or none. Such cells are probably caused by a delay in the first cleavage until preparations for the next have already begun. Fig. 11 shows an early stage of fission in a cell 8 × 7μ in which one of the daughter cells has two flagella and a haptonema, while the other has already formed two new flagella ready for the next fission. Such double-fission stages have been seen infrequently in individuals lacking chromatophores (Fig. 12), though such specimens are always smaller than those containing chromatophores, measuring 6–6·5 × 6·5–7μ.

A culture of this species when grown in Erdschreiber culture solution produces from 2 to 3 million motile cells per ml. at the peak of growth. After the peak of growth has been passed, for reasons which have not been analysed, the motile cells migrate to the bottom of the flask forming there a dark olive-green skin. The word migrate is used to express the fact that cells collect preferentially on one side of the flask showing a phototactic response. If this skin is scraped from the bottom and examined the non-motile stages illustrated in Figs. 13–18 are found. When the cells lose motility they become amoeboid, measuring about 7μ in diameter and, as far as can be checked by the use of cresyl blue, appear to discard their scale covering. At the same time small lobes appear at the periphery of the chromatophores (Fig. 13). Large naked (?) amoeboid stages up to 13 × 9μ are also found in which the chromatophores are so very finely lobed, and so pale in colour, that they are very difficult to distinguish (Figs. 14, 15). The pyrenoid-like bodies could be seen in some of these amoeboid stages, but they are smaller than in the motile phase. A few very small leucosin vesicles are also present in some of the cells. Ingested bacteria could be detected inside these cells and graphite particles were ingested.

The origin of the large amoeboid cells (Figs. 14, 15) has not been traced. They may be either the product of fusion of the smaller amoeboid forms (Figs. 13) or they may be double-fission stages in the non-motile phase. Stages, which are apparently early division stages of these large naked amoeboid forms (Fig. 15), show four chromatophores and eight pyrenoids. From the sizes of the different stages the large amoeboid cells must either contract considerably in size or else divide before they round off and develop a thin smooth wall. No large secondary mucilaginous envelope, as was found in the genera *Prymnesium* by Carter (1937) and Conrad (1941), and in *Isochrysis* and *Dicrateria* by Parke (1949), has been found surrounding the walled stages.

The smooth-walled cells with two or four pale chromatophores, stellate or finely lobed, divide to produce four walled daughter-cells (Figs. 16, 17). These daughter-cells become free from the parent membrane and usually have two chromatophores, but sometimes there appears to be only one; the chromatophores are still stellate or deeply lobed in form (Fig. 18). These daughter-cells could quite easily be mistaken for the free, non-motile, walled cells belonging to either the genus *Phaeaster* Scherffel (1927) or *Sarcinochrysis* Geitler (1930). The genus *Pleurochrysis*, recently described by Pringsheim (1955), differs from the non-motile phase of *Chrysochromulina* in not possessing finely lobed chromatophores, and in showing longitudinal divisions in the cells, to give short filaments which grow out to produce irregularly ramified colonies.

The swarmer of *Pleurochrysis* differs also in lacking a haptonema, and has either fine granules on the external membrane or is surrounded by a solid granulated capsule.

The reconstitution of the motile phase from the small, walled, Phaeaster-like cells (Fig. 18), which are ovoid to slightly bean-shaped and measure from $3{\cdot}5 \times 2{\cdot}5$ to $6 \times 4\mu$, has not yet been observed, but empty walls (Fig. 19) with a circular pore about $1{\cdot}5\mu$ in diameter have been found, suggesting that their contents are so liberated. It would be particularly interesting to watch the behaviour of the chromatophores immediately prior to and immediately after the liberation. As was stated earlier the chromatophores of the motile stage sometimes appear striated, when seen in face view, as if there might be lobes lying flat against the inner face.

In this species, if the olive brown skin on the bottom of a flask is washed well a number of times to remove all free cells and all motile cells and then fresh culture solution added, in 7–10 days the skin has gone from the bottom of the flask and a thick culture of the motile phase is then present in the medium.

Chrysochromulina minor n.sp., Parke & Manton

Diagnosis

Motile cells sphaeroidal to ovoid with a flattened flagellar pole and the opposite pole slightly tapering, some metaboly, 3–5 (exceptionally $2{\cdot}5$–$7{\cdot}5$)μ in diameter. Two equal homodynamic flagella, 2–3 times the cell diameter in length, with a distinct knobbed mucronate tip more than twice the flagellar diameter in length visible with the electron microscope. One haptonema[1] slightly shorter than the flagella when fully extended, arising with them at the flattened cell pole. Periplast covered by very thin sculptured scales, nearly circular to ellipsoid, sometimes hexagonal to octagonal, $0{\cdot}2 \times 0{\cdot}3$ to $0{\cdot}5 \times 0{\cdot}7\mu$ in size.

Cells uninucleate, no stigma. Chromatophores usually 1 or 2, sometimes 4, 8 or none, deep golden-brown, in motile phase saucer-shaped, parietal, with medium sized globular body (pyrenoid ?) on inner face placed near the margin nearest the flagellar pole; in non-motile phase deeply lobed or stellate. Oil and leucosin produced. Muciferous bodies localized near the non-flagellar pole. Nutrition holophytic and/or phagotrophic. Non-toxic to fish.

Asexual reproduction of pigmented and non-pigmented forms in motile phase by fission into 2 or 4 daughter-cells; in non-motile phase by fission of amoeboid cells to produce 4 walled daughter-cells which probably liberate motile phase.

Habitat: the sea at position Lat. N. 49° 19′, Long. W. 7° 26′ at 5 m. depth on 14 June 1950. Type culture (Plymouth No. 52) deposited with the Type Culture Collection, Cambridge, and the Marine Biological Association;

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[1] For derivation and use of this term see p. 581.

preserved material and photographs lodged with the Marine Biological Association, Plymouth, England.

Cellula motili sphaeroidali aut ovoidali, aliquanto planato ad axem qua inserta sint flagella, paululum subulata ad axem objectum flagellis, forma mutabili, 3μ ad 5μ (rare 2·5μ ad 7·5μ) latitudine. Duobus similibus flagellis homodynamicis, longioribus 2 aut 3 cellulae latitudine, utriusque ad extremitatem distincta regione nodosa et mucronata, longa plus bis obesitate flagelli, solum per microscopiam electronicam visibili; unico haptonemate, conjunctim flagellis exorienti, paulum breviore, cum maxime extensus sit, quam longitudo flagelli. Periplasto induto delicatissimis squamis sculptis, prope circularibus aut ellipsoidalibus, nonnunquam hexagonis aut octagonis, 0·2μ ad 0·5μ lat., 0·3μ ad 0·7μ long., visibilibus solum per microscopiam electronicam.

Nucleo unico, nullo stigmate; chromatophoris ex norma unico aut duobus, nonnunquam 4, 8 vel nullis, profunde aureofulvis, in cellulis status motilis crateriformibus, parietalibus, unocuique corpore globulari medio magnitudine (pyrenoidali?) in aspectu concavo locato ad marginem juxta axem qua oriantur flagella; in cellulis status non-motilis chromatophoris profunde lobatis aut stellatis; cellulis oleum leucosinumque parientibus. Corporibus muciferosis locatis ad regionem objectam axi flagellari. Nutritione holophytica et tamen phagotrophica. Non toxica piscibus.

Generanti asexualiter in statu motili per fissionem cellularum coloratarum vel incoloratarum in 2 aut 4 cellulas filiolas. In statu non-motili generanti per fissionem amoeboidalium cellularum ad 4 cellulas filiolas, parietibus praeditas, generandas, quibus potest ut cellulae status motilis liberentur.

Habitat mare ad locationem Lat. N. 49° 19′, Long. W. 7° 26′, profund. 5 m. (14 Jun. 1950).

Description

This species is smaller and usually slightly more ovoid in shape than *C. kappa* when alive (Fig. 36), though this difference is obscured after fixation. In an actively growing culture 75% of the cells are from 3 to 5μ in length or diameter, 5% are 2–3μ and the remaining 20% are early fission stages (including double-fission stages) from 5 to 7·5μ. The flagella, 2–3 times the length of the cell, are relatively slightly longer than in *C. kappa* and are more easily seen, though they are more readily thrown off when the species is examined under the light microscope. No bulbous swelling can be detected at the base of the haptonema with the light microscope in this species though a comparable structure can be demonstrated with some difficulty with the electron microscope. The length of the haptonema when fully extended is slightly less than that of the flagella, thereby differing from the situation in *C. kappa* in which it is slightly more. The club-shaped extremity is similar in the two species but the translucent haptonemal sheath is slightly less conspicuous in *C. minor* (Fig. 60, Pl. VI).

The scales (Figs. 62–4, Pl. VII) are very similar in the two species, although we have so far failed to find any trace of spined scales in *C. minor*. The chromatophores of the motile phase are very similar to those of *C. kappa*, but are more deeply pigmented and more frequently show striations; they are usually 1 or 2 but sometimes 4, 8 or none. Individuals lacking chromatophores, seen frequently in this species, measure usually 2·5–3μ (Fig. 40). When one chromatophore is present it lies laterally (Fig. 36); when about to divide it moves to the non-flagellar pole (Fig. 39) and after division the two chromatophores return to the lateral position (Fig. 38).

In this species the pyrenoid-like bodies (p. 589), reacting in the same way to stains and Millon's reagent as in *C. kappa*, are smaller and are excentrically placed near to the periphery of a chromatophore on the side towards the flagellar pole (Figs. 36–38), not centrally on its inner face as in that species. The position of the nucleus, leucosin vesicles, oil globules and muciferous bodies are as in *C. kappa* except that the muciferous bodies are fewer and none seem to be present at the flagellar pole. Their position at the opposite pole changes, as in *C. kappa*, with the metaboly of the body. The behaviour of the muciferous bodies is similar in both species.

Swimming is more rapid than in *C. kappa* and tends to be in straight lines over considerable distances rather than moving in circles. As in *C. kappa* the most usual attitude, and the only one associated with rapid swimming is with the flagellar pole and appendages directed backwards, but the flagella lie closer together so that their ends are separated by less than the width of the cell (Fig. 36). The attitudes of the haptonema are the same as those described for *C. kappa*, both when projecting backwards and when projecting forwards, but in the latter position the flagella lie more nearly in front of the body than in

Legends to Text-figs. 36–44

Chrysochromulina minor n.sp. (× 5000).

Fig. 36. Individual swimming with flagella and haptonema behind the body, in the position characteristic for the species during rapid swimming. *c*, chromatophore; *f*, flagellum; *h*, haptonema; *l*, leucosin vesicle; *m*, muciferous body; *n*, nucleus; *p*, pyrenoid-like body; *s*, scales.

Fig. 37. Anchored individual with 2 chromatophores and very small masses of ingested graphite near flagellar pole.

Fig. 38. Anchored individual ingesting minute particles of graphite at non-flagellar pole; cell body swaying round in circles in a clockwise direction from anchoring point.

Fig. 39. Individual swimming with flagella and haptonema in front of the body, haptonema fully extended; ingested graphite (*g*) towards flagellar pole; chromatophore moved from lateral position to pole prior to division.

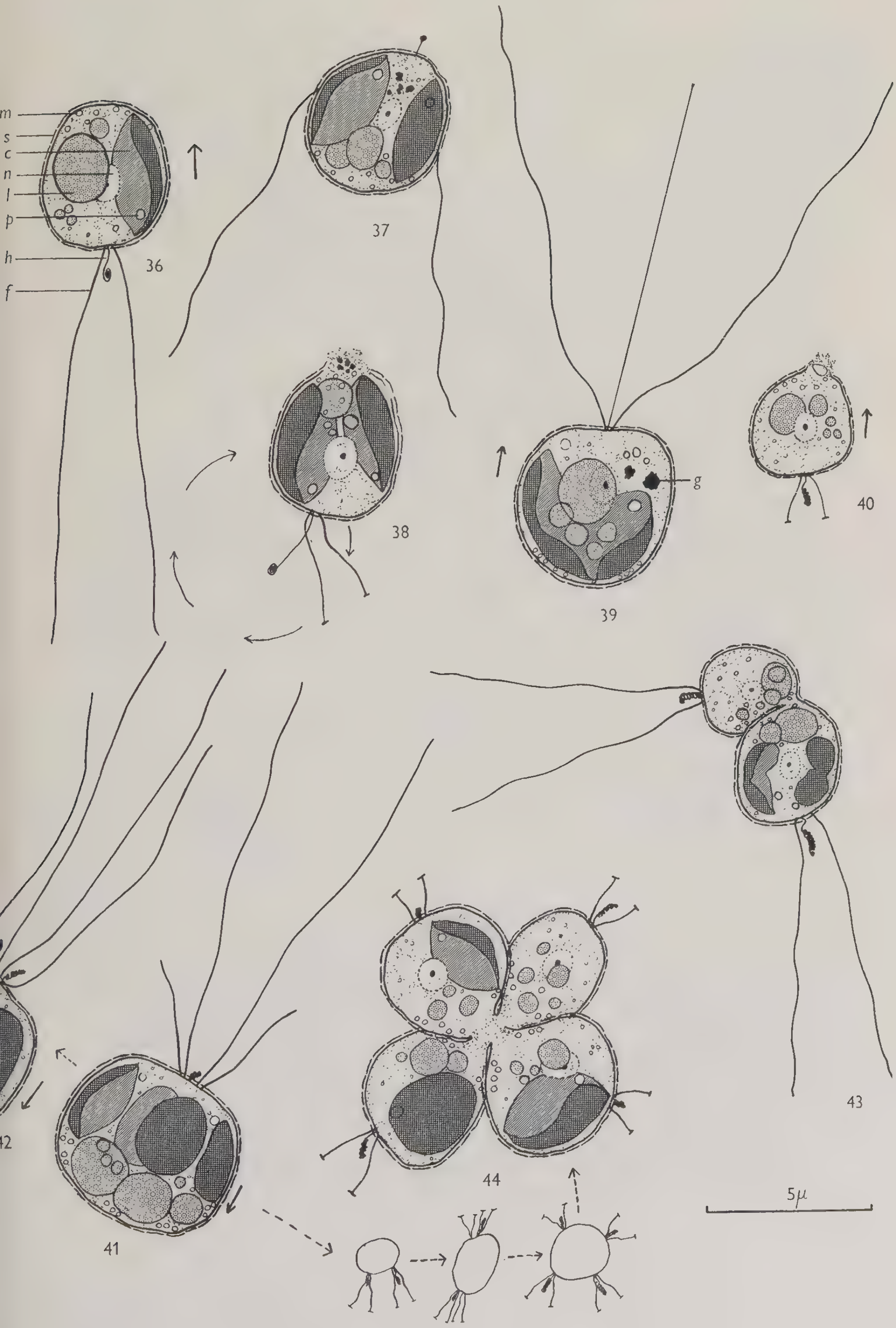
Fig. 40. Swimming individual lacking chromatophores, ingesting a bacterium at non-flagellar pole.

Fig. 41. Early fission stage with 4 chromatophores and two new flagella just developing; second haptonema not yet formed.

Fig. 42. Flagellar pole of individual in early fission stage; two new flagella nearly as long as original flagella and second haptonema developed.

Fig. 43. Two motile daughter-cells just before separation after unequal fission; larger daughter-cell with two dividing chromatophores and smaller without chromatophores.

Fig. 44. Double-fission practically completed giving 4 daughter-cells, one of which lacks a chromatophore; earlier stages shown diagrammatically below.


m
s
c
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l
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h
f
36
37
38
g
39
40
42
41
44
43
5μ

Text-figs. 36–44.

C. kappa. Movement of the cell with the appendages directed forwards is, however, less frequently seen in this species than in *C. kappa*.

A fairly marked phototactic response is shown by this species also, in spite of the absence of an obvious stigma. The method of attachment (Figs. 37–38) of the haptonema is as described on p. 591.

Phagotrophy is less conspicuous here than in *C. kappa* since the particles ingested are usually much smaller (Figs. 54–55, Pl. V; Figs. 37–38). Bacteria (Fig. 40) and graphite up to about 0·5μ (Figs. 54–55, Pl. V; Fig. 39) are, however, readily ingested in the manner described on p. 592, but only occasionally an individual is seen containing anything as large as 1 μ (Fig. 53, Pl. V). Under an oil-immersion lens very minute graphite particles can be seen in Brownian movement within vacuoles and frequently a number of separate particles (Fig. 54, Pl. V; Figs. 37–39) may be present in one cell. Ingested material in this species, unlike *C. kappa*, tends to be moved within the body towards the flagellar pole, i.e. away from the point of entry or possibly towards the pyrenoid-like bodies (Figs. 37, 39).

Fission in the motile stage is similar to that described for *C. kappa*; it can be equal or unequal, and when unequal the smaller daughter-cell is frequently colourless (Fig. 43). Double-fission stages, 6–7·5μ in diameter, are seen much more frequently in this species than in *C. kappa*. They can be quite sphaeroidal and rotate with a kind of rolling movement. Colourless daughter-cells are also produced from the double-fission stage (Fig. 44). The growth of the new flagella was observed in this species; they arise on either side of the original pair (Fig. 41) and as they increase in length the second haptonema arises (Fig. 42).

This species behaves similarly to *C. kappa* in forming a skin on the bottom of the flask after the peak of growth has been reached (6 to 7 million cells per ml.), and from this skin stages similar to those found in *C. kappa*, from the amoeboid cells (Fig. 45) to the small, walled daughter-cells (Fig. 51), have been obtained. The large amoeboid cells, which have been seen to ingest masses

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Legends to Text-figs. 45–52

Chrysochromulina minor n.sp. (×5000).

Fig. 45. Amoeboid individual showing 2 deeply lobed pale chromatophores (*c*), two pyrenoid-like bodies (*p*), and ingested graphite masses (*g*).

Fig. 46. Large walled cell with two chromatophores.

Fig. 47. Early stage of 1st fission of a large, walled cell.

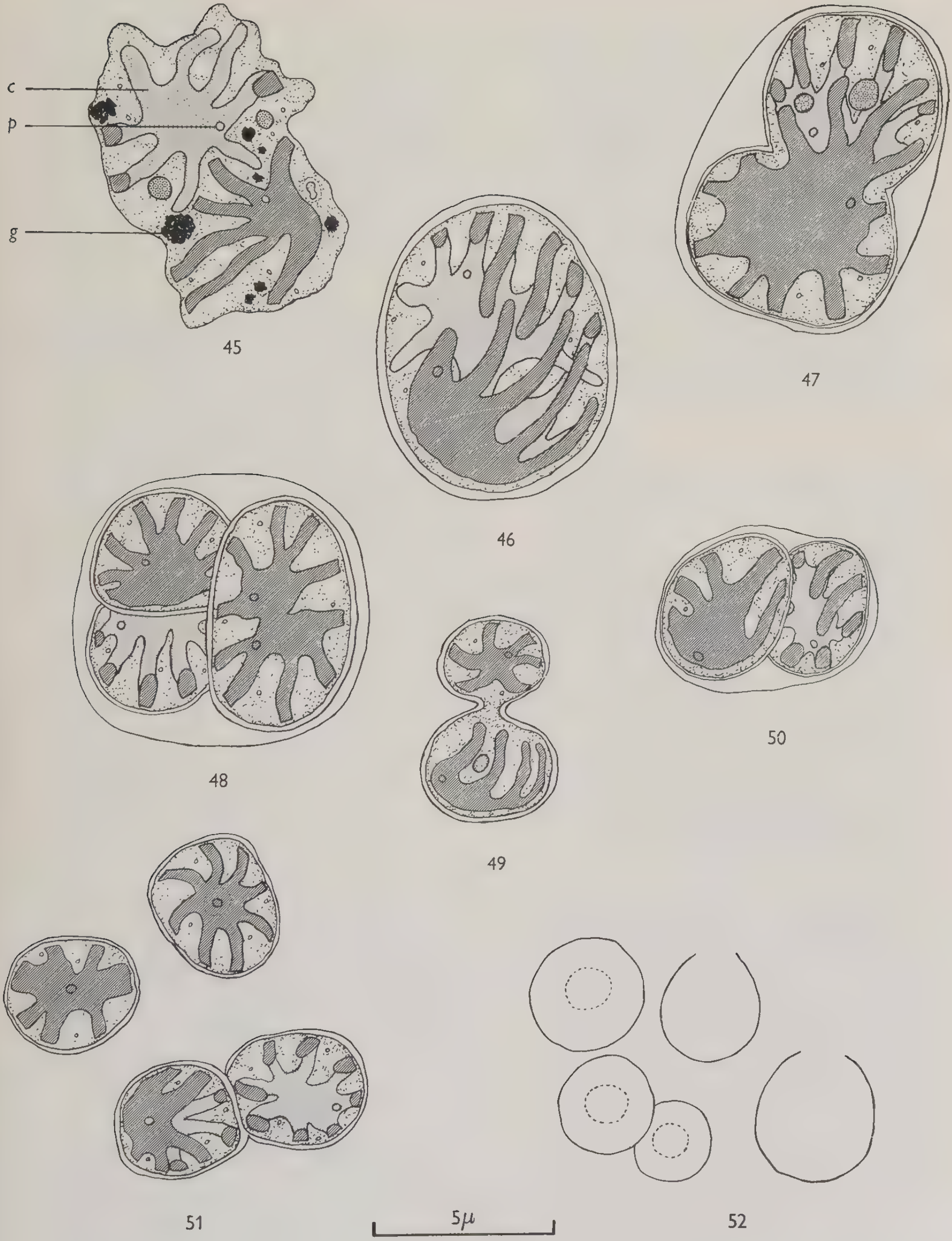
Fig. 48. Second fission partly completed but still inside parent membrane.

Fig. 49. Unequal second fission; cell free from parent membrane.

Fig. 50. Equal second fission; cell free from parent membrane.

Fig. 51. Four walled daughter-cells, product of second fission, each with one stellate or deeply lobed chromatophore and one pyrenoid-like body.

Fig. 52. Empty walls of daughter-cells showing size variation and pore through which motile stage has probably been liberated.


c
p
g
45
46
47
48
49
50
51
5μ
52

Text-figs. 45–52.

of graphite (Fig. 45), measure from 4 × 8 to 5·5 × 8·5 μ. As in the previous species the chromatophores in these stages are very pale and finely lobed (Fig. 45–51). The large, walled cells, usually ovoid, measure from 4·5 × 7·5 to 6 × 8 μ (Fig. 46) and fission stages of them (Figs. 47–51) have been obtained. In this species the products of the first fission (Fig. 47) can either remain in the parent membrane (Fig. 48) or become free (Figs. 49, 50). The second fission can be equal or unequal (Figs. 49, 50). The walled daughter-cells, product of the second fission, vary considerably in shape and size (Fig. 51); they can be sphaeroidal to ovoid or somewhat angular and measure from 2 × 2·5 to 3 × 4 μ; the chromatophores are still distinctly stellate or very deeply lobed. The empty walls from which the motile stage has apparently been liberated (Fig. 52) show also a considerable variation in size, the pore through which the cell has been liberated measuring about 1 μ. In this species when fresh culture

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Explanation of Plates V–VII

Chrysochromulina minor n.sp.

V

Fig. 53. A cell photographed in a sealed liquid mount after osmic vapour killing, to show the effect of graphite feeding; an abnormally large pellet of ingested graphite is visible between the two chromatophores. Visual light, oil-immersion objective, magnification × 2000.

Fig. 54. A similar cell with two small graphite pellets of a size more usual in this species.

Fig. 55. A similar cell showing two patches of ingested graphite and also the two flagella and coiled haptonema.

Fig. 56. A group of cells killed with iodine in KI (in sea water) and dried on glass. Photographed dry with visual light, magnification × 1000.

Fig. 57. Part of the same group of cells as the preceding stripped from glass and remounted for the electron microscope. Electron micrograph M. 160·1, gold palladium shadowing 60 kV, magnification × 3000.

Fig. 58. More highly magnified view of the lowest cell of Fig. 57. Electron micrograph M. 160·2, 60 kV. magnification × *c.* 6000.

VI

Fig. 59. Two cells transferred from glass to show coiled haptonemata. Electron micrograph M. 160.7, 60 kV, magnification × 5000.

Fig. 60. Part of a cell killed directly on the formvar film, showing the coiled haptonema with its sheath more clearly. Electron micrograph M. 174·17, 60 kV, magnification × 20,000.

Fig. 61. Detail of the tip of a flagellum to show the long attenuated point with slight terminal swelling (see also Fig. 58, Pl. V). A detached scale and the basal part of a *Caulobacter* (cf. Fig. 28, Pl. II) are visible in the middle of the field. Electron micrograph M. 203·26 taken on the Philips microscope at Messrs Tootals of Manchester, 60 kV, magnification × 15,000.

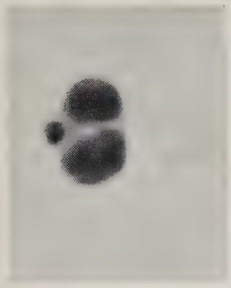
VII

Fig. 62. Part of a cell killed directly on the formvar, more highly magnified to show scales and the bases of the three appendages. Electron micrograph M. 174·11, 60 kV, magnification × 20,000.

Fig. 63. Reversed print of part of the preceding, more highly magnified to show the details of sculpturing of the scales. Electron micrograph M. 174·11, magnification × 30,000.

Fig. 64. Scales from another specimen showing raised rims more clearly. Electron micrograph M. 174·5, 60 kV, magnification × 30,000.

53



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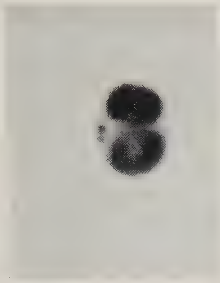

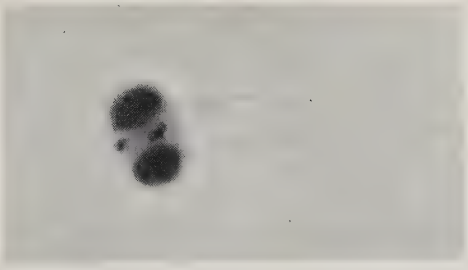

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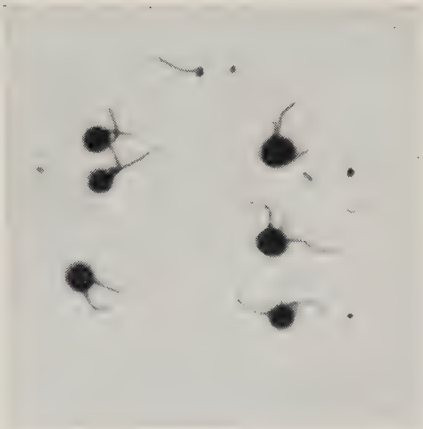

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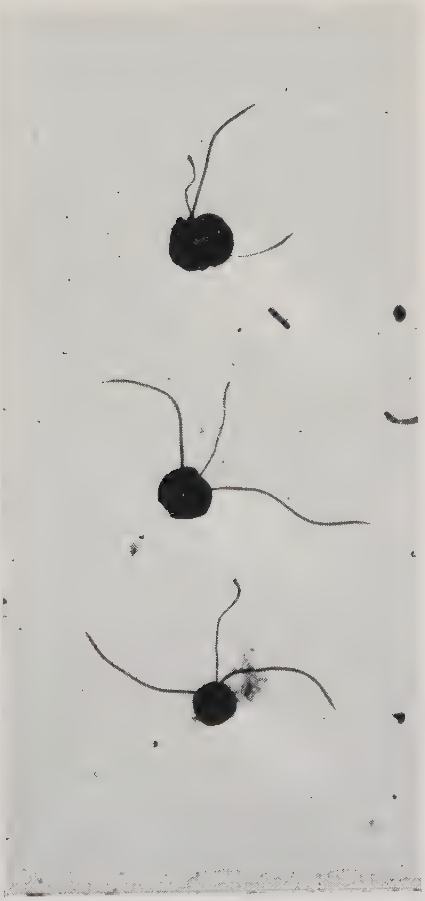

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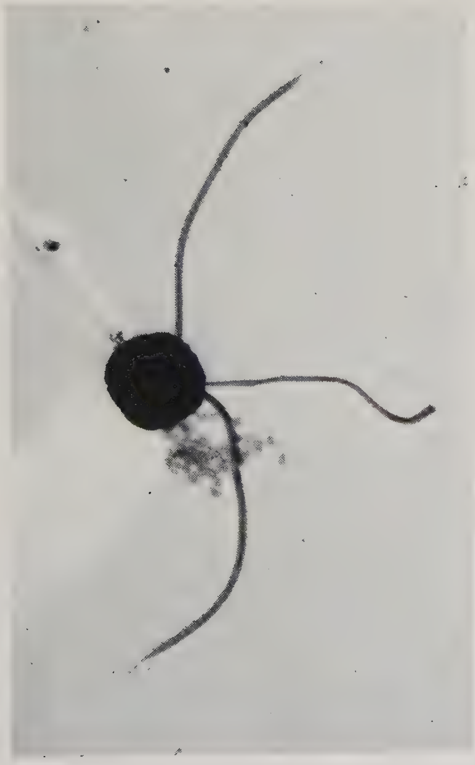

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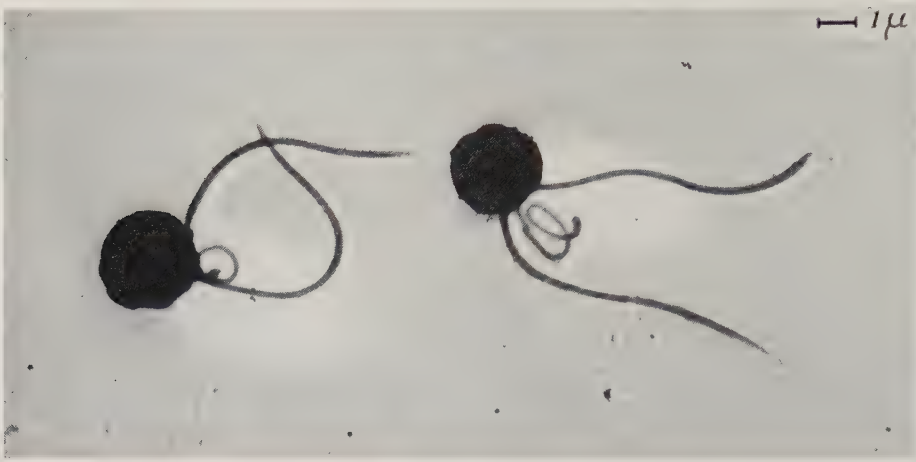
(*Facing p.* 600)



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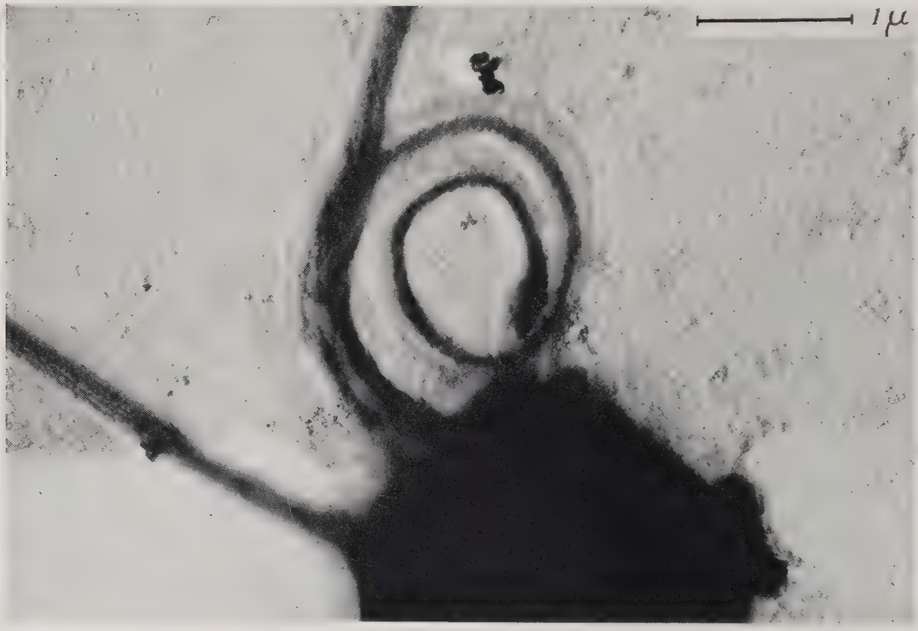

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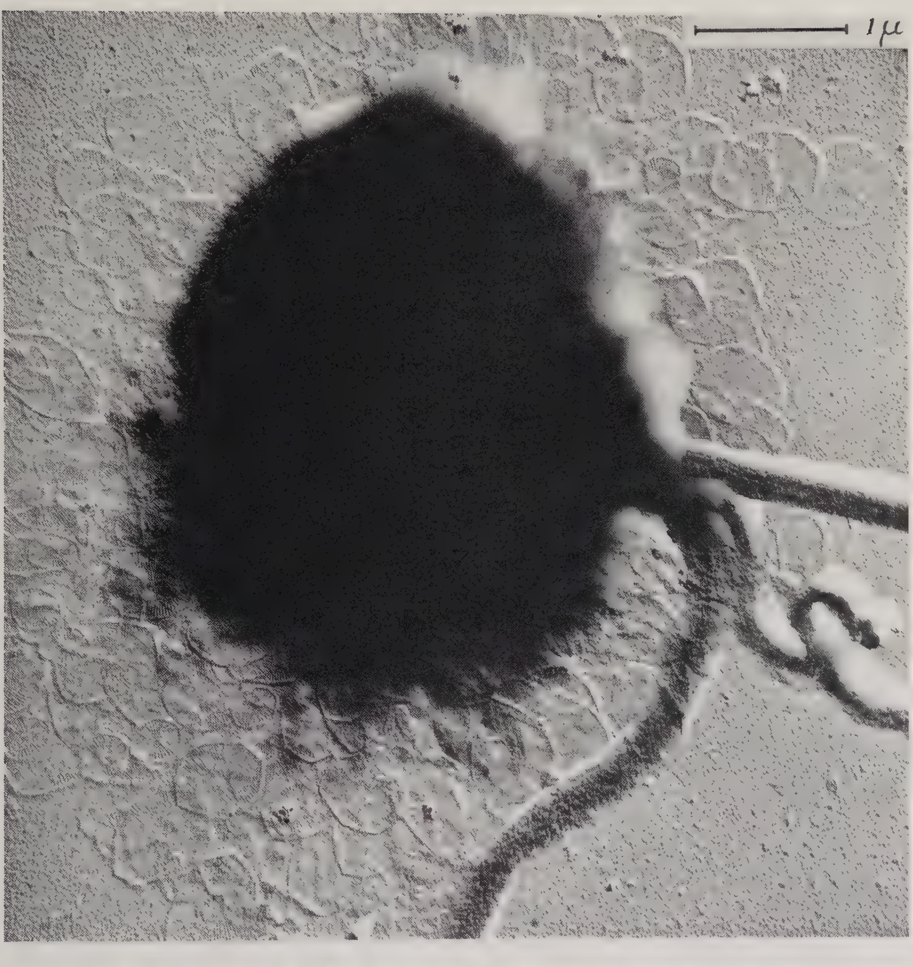

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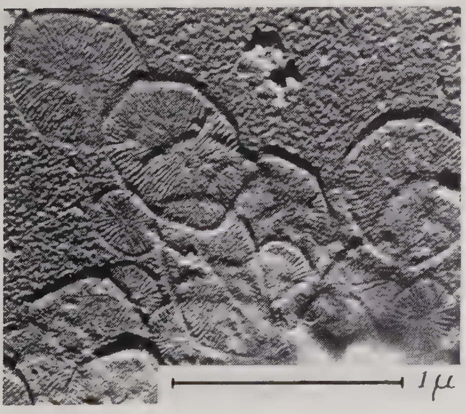

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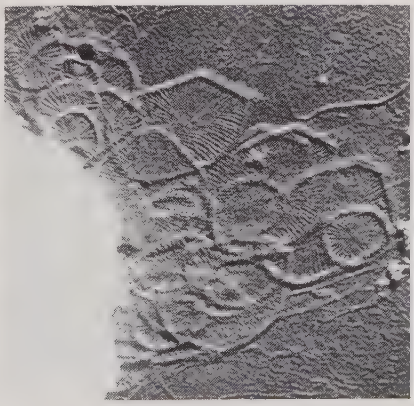

64

solution is added to the skin on the bottom of a flask, after it has been washed, the motile phase is quickly produced, much more rapidly than in *C. kappa*, so that in 3–4 days a fairly thick culture of the motile stage is obtained.

Chrysochromulina brevifilum n.sp., Parke & Manton

Diagnosis

Motile cells sphaeroidal to pyriform with flagellar pole usually flattened; metaboly of body well-marked particularly at non-flagellar pole; cells 4–7μ (exceptionally 3·5–11·5μ) in length or diameter. Two equal, homodynamic, shortly mucronate flagella, 2½–3½ times cell diameter in length and one haptonema,[1] 2–2½ times cell diameter in length when fully extended, arising close together at flattened pole. Periplast covered by very thin sculptured ellipsoid scales, approximately 0·7μ across, each with a central spine attached by four decurrent ridges, spine length approximately equal to scale diameter.

Cells uninucleate, no stigma. Chromatophores usually 2 or 4, occasionally 1 or 8, golden brown; in motile phase saucer-shaped, ellipsoid or oblong, parietal with small globular body (pyrenoid ?) on inner face of each near the margin towards the non-flagellar pole; in non-motile phase deeply lobed or stellate. Oil and leucosin produced. Muciferous bodies conspicuous, usually in rows, but their position changing with the metaboly of the body. Nutrition holophytic and/or phagotrophic. Not toxic to fish.

In motile phase asexual reproduction by fission into 2 or 4 daughter-cells: in non-motile phase by fission of amoeboid cells to produce 4 walled daughter-cells which probably liberate motile phase.

Habitat: the sea at position Lat. N. 49° 21′, Long. W. 04° 54′ at surface on 9 May 1950.

Type culture (Plymouth No. 39) deposited with the Type Culture Collection, Cambridge, and the Marine Biological Association: preserved material and photographs lodged with the Marine Biological Association, Plymouth, England.

Cellula motili, sphaeroidali vel pyriformi, ex norma planata ad axem qua inserta sint flagella; forma manifeste mutabili imprimis ad axem objectum flagellis; cellula 4μ ad 7μ (rare 3·5μ ad 11·5μ) latitudine. Duobus similibus flagellis homodynamicis, breve mucronatis, longioribus 2½ ad 3½ cellulae latitudine; unico haptonemate conjunctim flagellis exorienti, longiore, cum maxime extensus sit, 2–2½ cellulae latitudine. Periplasto induto delicatissimis squamis sculptis ellipsoidalibus, prope 0·7μ latitudine, unaecuique squamae spinulo centrali unico per quattuor juga decurrentia affixa; spinuli longitudine subaequali squamae latitudini, visibilibus solum per microscopiam electronicam.

Nucleo unico, nullo stigmate; chromatophoris ex norma 2 aut 4, nonnunquam unico aut 8, aureo-fulvis; in cellulis status motilis chromatophoris crateriformibus, ellipsoidalibus aut oblongis, parietalibus, unocuique chromatophoro parvo corpore globulari (pyrenoidali?) locato in aspecto interiore ad marginem remotam ab axi qua inserta sint flagella; chromatophoris profunde lobatis aut stellatis in cellulis status non-motilis. Cellulis oleum leucosinumque parientibus. Corporibus muciferosis ex norma conspicuosis, plerumque in ordinibus instructis, sed locatione variosa secundum mutationem formae cellulae. Nutritione holophytica et tamen phagotrophica. Non toxica piscibus.

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[1] For derivation and use of this term see p. 581.

Generanti asexualiter in statu motili per fissionem in 2 aut 4 cellulas filiolas; in statu non-motili generanti per fissionem cellularum amoeboidalium ad cellulas filiolas, parietibus praeditis, producendas, quibus potest ut cellulae status motilis liberentur.

Habitat mare ad locationem Lat. N. 49° 21′, Long. W. 04° 54′ ad summum mare (9 Mai 1950).

Description

In the motile phase this species is larger than *C. minor* (Figs. 73, 75, 77, Pl. VIII) and shows greater variation in cell-shape caused by the more pronounced metaboly of the body (Figs. 65–69) than either *C. kappa* or *C. minor*. In an actively growing culture, excluding cells in which fission has already started, 77% of the cells are from 4–7μ in diameter or length, 7% are from 3·5 to 4·0μ and the remaining 16% are early fission stages (including double-fission stages) from 7 to 11·5μ.

The flagella, $2\frac{1}{2}$–$3\frac{1}{2}$ times the body diameter in length, are fairly robust and can be seen quite clearly under the light field, although they tend to be thrown off from the body under light and dark ground fields even more rapidly than those of *C. minor*. The haptonema also shows up very clearly under the light

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Legends to Text-figs. 65–72

Chrysochromulina brevifilum n.sp. (× 5000).

Fig. 65. Individual swimming with flagella and haptonema behind body, in the position characteristic for the species during rapid swimming; chromatophores dividing. *c*, chromatophore; *f*, flagellum; *h*, haptonema; *l*, leucosin vesicle; *m*, muciferous body; *n*, nucleus; *p*, pyrenoid-like body; *ss*, spined scales.

Fig. 66. Anchored cell swaying round in circles on nearly fully extended haptonema; chromatophores broadening preparatory to division, 4 pyrenoid-like bodies; small mass of graphite just ingested at non-flagellar pole.

Fig. 67. Individual anchored by haptonema which is hardly extended; cell elongated by metaboly: large mass of ingested graphite (*g*) at non-flagellar pole.

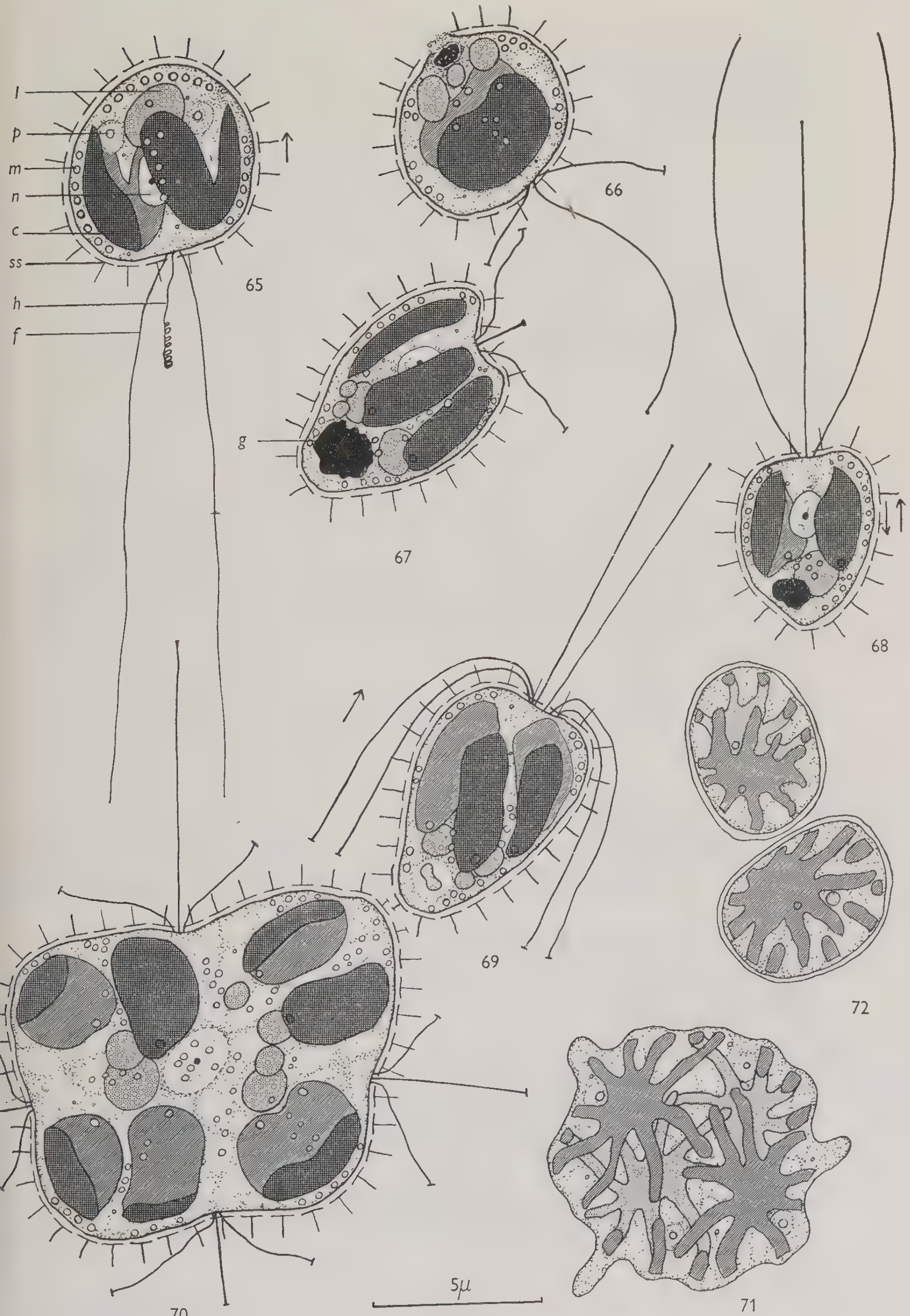
Fig. 68. Individual swimming with flagella and haptonema in front of body, haptonema fully extended; ingested graphite at non-flagellar pole.

Fig. 69. Early fission stage: 4 flagella, 2 haptonemata fully extended, body elongated and chromatophores displaced by metaboly; an ingested bacterium in a vacuole at non-flagellar pole.

Fig. 70. Stage with four pairs of flagella and four fully extended haptonemata developed prior to double-fission.

Fig. 71. Large amoeboid individual with four deeply lobed pale chromatophores and four pyrenoid-like bodies.

Fig. 72. Two walled daughter-cells from which motile stage is probably liberated; each with two pale stellate chromatophores.


l
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m
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65
66
g
67
68
69
70
71
72
5μ

Text-figs. 65–72.

microscope and when fully extended varies in length generally from $1\frac{1}{2}$ to 2 times the cell length (Figs. 73, 75, 77, Pl. VIII; Fig. 68); if, however, the cell has become exceptionally elongated by metaboly then the fully extended haptomena is not very much longer than the actual cell length (Fig. 69). No bulbous swelling has been detected at the proximal end of the haptonema (Figs. 73, 75, Pl. VIII) and its delicate sheath covering appears not so wide nor so obvious as in the other two species (Figs. 78, 79, Pl. IX); the slightly swollen club-shaped distal end is shown very clearly in Fig. 78, Pl. IX.

The spined surface scales of the cell can be seen in Figs. 78–81, Pl. IX, the position of the spines being outwards from the body (Figs. 78–79, Pl. IX). The spines are better defined and stronger than those on the few polar scales of *C. kappa* though they are of the same general type. The scale itself is ovoid to polygonal in outline, with a raised margin and a system of fine sculptural ridges on the surface; the spine is subtended by four powerful, buttress-like decurrent ridges.

In the motile phase the chromatophores are not so deeply pigmented as in *C. kappa* and *C. minor* and tend to be more elongated in shape and to lie in the body closer to the flagellar pole than in the two previous species (Figs. 65, 67). Their shape and position can change very considerably with the metaboly of the body. There are usually 2 (Figs. 65, 66), or 4 (Figs. 67, 69), occasionally 1 or 8. As in *C. kappa* and *C. minor* they often appear striated. No individuals lacking chromatophores have so far been observed in *C. brevifilum*. The

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Explanation of Plates VIII and IX

Chrysochromulina brevifilum n.sp.

VIII

Fig. 73. Two cells killed with iodine as in Fig. 56, Pl. V, photographed dry on glass. Visual light, magnification × 1000.

Fig. 74. Another cell of the same showing a division stage. Visual light, magnification × 1000.

Fig. 75. The two cells of Fig. 73 stripped from glass and examined with the electron microscope after gold palladium shadowing. Electron micrograph M. 185·1, 60 kV, magnification × 3000.

Figs. 76, 77. Two focal levels of one rather large cell showing an ingested graphite pellet, several chromatophores and parts of the three appendages. Visual light photograph from a liquid mount made after killing with osmic vapour, oil-immersion objective, magnification × 2000.

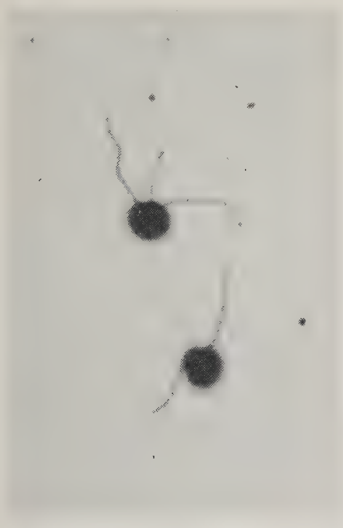
IX

Fig. 78. Part of a cell killed directly on the formvar film showing the haptonema. Electron micrograph M. 150·17, gold palladium shadowing, 60 kV, magnification × 5000.

Fig. 79. A similar cell more highly magnified showing spined scales in position and part of the coiled haptonema with its sheath. M. 150·20, 60 kV, magnification × 10,000.

Fig. 80. Reversed print of scales from the lower cell of Fig. 75, Pl. VIII. Electron micrograph M. 183·4, 60 kV, magnification × 20,000.

Fig. 81. Detail of scales from the cell of Fig. 74, Pl. VIII, after transfer from glass. Electron micrograph M. 183·10, 60 kV, reversed print, magnification × 20,000.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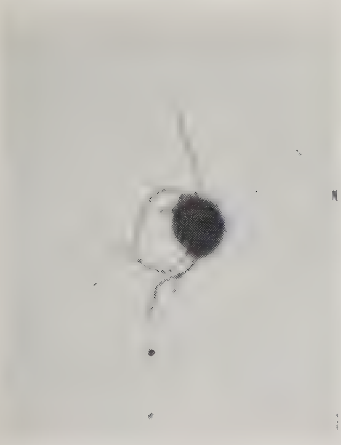
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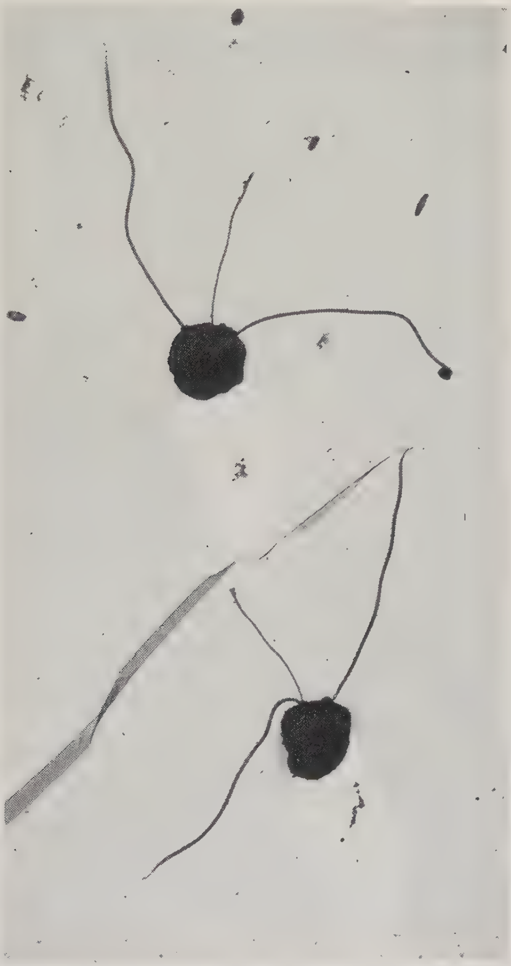
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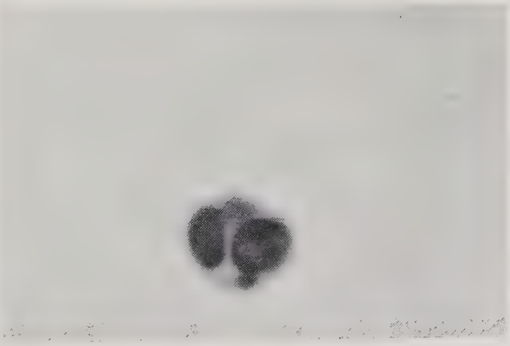
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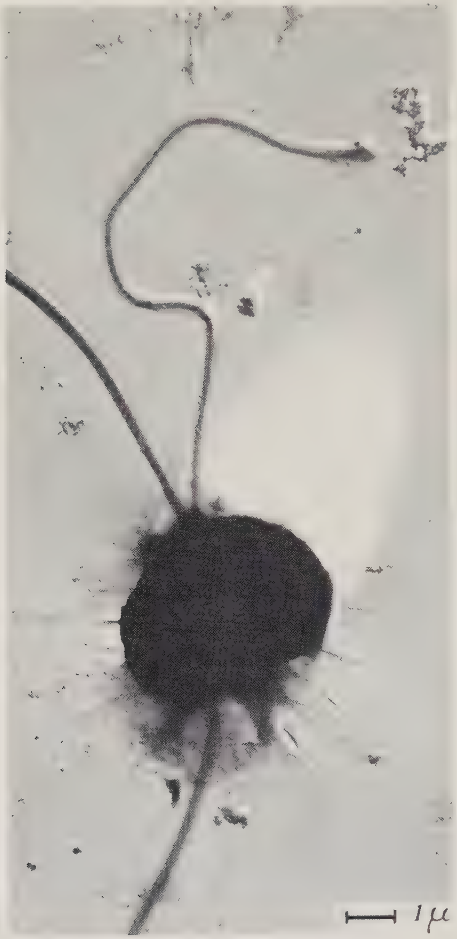

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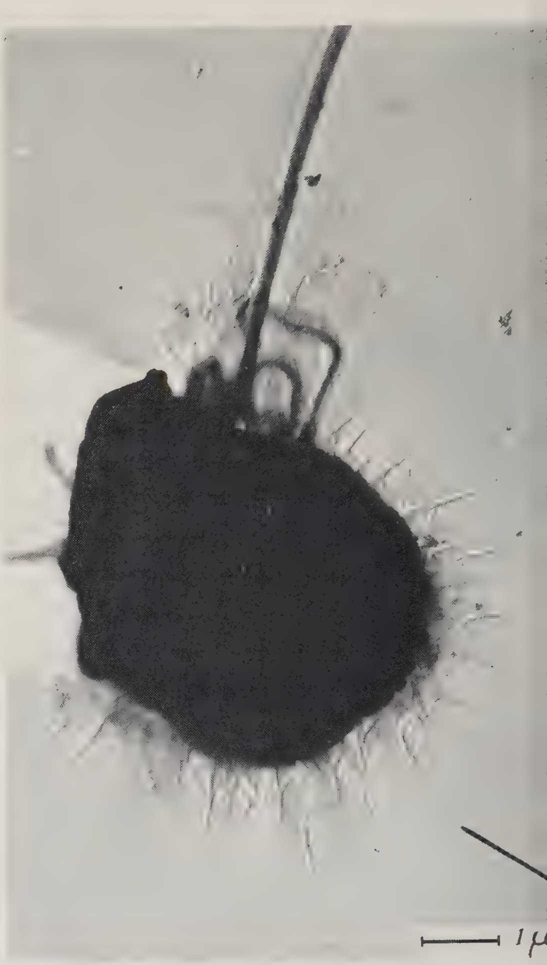

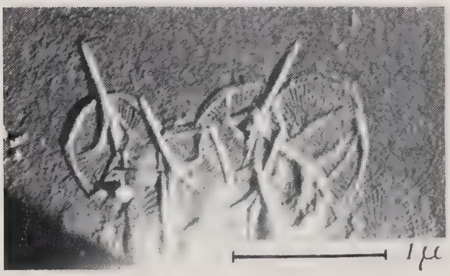

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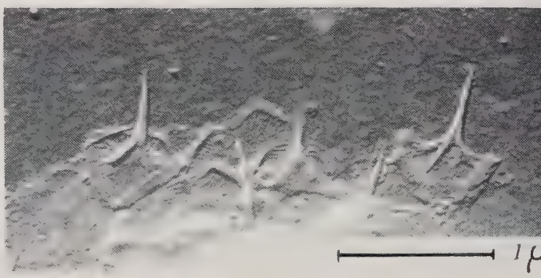

pyrenoid-like bodies also react in the same way to stains and Millon's reagent as do those of *C. kappa* and *C. minor*. They are small and are excentrically placed near to the periphery of a chromatophore, as in *C. minor*, but in *C. brevifilum* they lie on the side away from the flagellar pole (Figs. 65–69). They are frequently surrounded by non-refringent material (Fig. 65).

The position of the nucleus, leucosin vesicles and oil globules is as in *C. kappa* and *C. minor*. The muciferous bodies are much larger and more conspicuous in *C. brevifilum* than in the two previous species. When the cell body is more or less sphaeroidal they appear to lie in the peripheral cytoplasm in four rows, running from the flagellar pole to the opposite pole, with additional ones at the non-flagellar pole (Fig. 65). Their position, however, does not remain constant but changes with the metaboly of the body (Figs. 66–69). When they discharge their contents rapidly, short rods are ejected, but if discharge is slow the contents ooze out to form a balloon which eventually becomes detached from the cell as a sphere which can remain visible for some considerable time.

In this species granules, possibly mitochondria, lying in the same position as the muciferous bodies, again stain bright blue with cresyl blue.

As in the two previous species, the flagella and haptonema are usually directed backwards when the cells are moving most rapidly; the flagella are not splayed out from the body as in *C. kappa*, but lie rather close together (Fig. 65) as in *C. minor*; the haptonema can be coiled up close to the body, partly extended, or sometimes, when movement slows down, even wholly extended straight out behind the body.

This species does not swim very rapidly. It has a slowish rolling movement rotating and gyrating, moving only for short distances; it then slows down, almost stops and in a very short time shoots off again with almost a jump usually in a different direction. This jumping type of movement is seen frequently: a cell jumps or jerks a short distance, stops, then jumps again and stops and can continue doing this for several minutes. This type of action is probably connected with a sweeping movement of the flagella characteristic of this species. The flagella lying either out from the body, or close to the body (Fig. 69), appear to stiffen suddenly and they are then brought close together into the position shown in Fig. 68 by a quick sweeping movement. This quick movement causes the cell to jump. Swimming with the flagella and haptonema in front of the body (Figs. 68–69) is seen more often than in *C. kappa* and *C. minor*. As in the two previous species, a marked phototactic response is shown although again there is no obvious stigma.

Phagotrophy occurs commonly in *C. brevifilum*. The cells readily ingest bacteria or graphite up to a size of 2μ, occasionally up to $2{\cdot}5\mu$, in the same manner (Figs. 76, 77, Pl. VIII; Figs. 66–69) as described for *C. kappa*; small cells $4{\cdot}5\mu$ in length have been found to contain graphite masses as large as 2μ in diameter. In this species the ingested material always remains very close to the non-flagellar pole (Figs. 66–69) and apparently is not moved about inside the body as in *C. minor*.

Temporary attachment of the cells by means of the haptonema (Figs. 66, 67) takes place in a similar manner to that described for *C. kappa*. The usual dancing of the body occurs when the attached haptonema is very little extended (Fig. 67) and the cell, as in the previous species, could then be mistaken for a *Prymnesium*. The exact details of configuration of the coiled part of an attached haptonema are more difficult to determine than in the two previous species as the body usually obscures the whole organ; when seen, however, it appears to be coiled in a flat spiral as described on p. 591. When the attached haptonema is fully extended (Fig. 66) the cell can frequently be seen to swing round in a circle from the central anchoring point.

Fission in the motile stage (Fig. 74, Pl. VIII) can be equal or slightly unequal and is similar to that described for the two previous species, except that here the two new flagella and the second haptonema tend to be formed before there is any broadening of the cell preparatory to the actual fission (Fig. 69). Double-fission stages (Fig. 70), 8·5 × 10·5 to 9·0 × 11·5μ, are seen much less frequently in this species than in *C. kappa* and *C. minor*. When seen, however, the four haptonemata are sometimes fully extended as shown in Fig. 70.

As in the two previous species the motile phase, after the peak of growth has been reached (1 to 2 million cells per ml.), eventually settles on the bottom of the flask and passes into the amoeboid phase, the cells of which develop finely lobed, very pale chromatophores (Fig. 71). In this species, as in *C. minor*, the products of the first fission can either remain in the parent membrane or become free. The second fission is usually equal, but can be slightly unequal. The walled daughter-cells (Fig. 72), produced by this fission, are usually ovoid with one side rather flattened; they range in size from 2·5 × 3 to 4 × 5μ. Empty walls from which the motile stage probably has been liberated have so far not been seen in this species. As in *C. kappa* and *C. minor*, if fresh culture-solution is added to a flask containing only the non-motile stage, the motile stage is produced and the skin disappears at approximately the same rate as in *C. kappa*.

DISCUSSION

It is obvious that many details of structure, behaviour and life history are still uncertain in all of these species, some of which can be clarified only by much further work. A conspicuous omission, which we hope shortly to remedy, is a report on the micro-anatomy of the cells and their appendages by means of thin sections and the electron microscope. Had we delayed the present description until this side of the programme could be completed, however, it would have held up publication of existing facts inconveniently long, at the risk also of producing a communication too large for publication in one piece.

On the experimental side we have not so far succeeded in observing the actual process of production of the motile phase from the dormant non-motile phase, nor have we demonstrated any process of sexual reproduction in these forms though negative evidence does not exclude it. There is indeed suggestive evidence, from the apparently invariable production of the small, walled daughter-cells in fours that here, if anywhere, is the place to look for some more direct indication of either a sexual fusion or a meiotic process without which these stages cannot be further interpreted.

With regard to that most characteristic organ, the *haptonema*, we can by no means yet specify its full biological significance in spite of the care with which its behaviour has been examined. That the detection of phagotrophic feeding was an immediate consequence of this examination can perhaps too easily suggest that there is a causal connexion. Such a suggestion admittedly is strengthened by finding that phagotrophic feeding is in fact practised by all our new organisms in which a haptonema has been demonstrated, and it was also reported in the analogous case of *Prymnesium saltans* by Conrad (1941). We cannot, however, state that a haptonema or analogous organ is essential to such feeding since this is also practised by the non-motile amoeboid stages and by other flagellates lacking the organ, notably some freshwater species of *Ochromonas* (Lund, 1942; Pringsheim, 1952) and *Chromulina* (Hutner & Provasoli, 1951). The reasons for the apparent association of a haptonema with phagotrophic feeding in the marine plankton are therefore not as self-evident as might have been expected and such description as we have so far been able to give of its behaviour has not yet provided the complete answer.

The formidable task of a biochemical analysis of the nutritional needs with respect to which phagotrophy is practised has been outside our scope of inquiry, though it would obviously be a matter of the very greatest interest if it could be undertaken as successfully as in recent work on *Ochromonas* (Pringsheim, 1952; Hutner, Provasoli & Filfus, 1953; Hutner & Provasoli, 1955). Another field in which we still lack information concerns the relative frequency of these organisms in the sea. We hope at a later stage, when a greater number of the commonest flagellates have been isolated and described, to attempt to obtain some quantitative figures for their frequency. At present, however, the undescribed species are so numerous that a quantitative estimate of individual species is scarcely obtainable. The most that we can state is that this type of organism is abundant both in species and in individuals, previous failure to detect them being solely due to their fragility and not at all to their rarity. In the total metabolic turnover in the plankton as a whole, therefore, they cannot fail to be playing an integral and perhaps an important part.

SUMMARY

Diagnoses and descriptions are given of three new species of marine plankton flagellates in the class Chrysophyceae. All possess two equal, homodynamic flagella and a third filiform appendage to which the name *haptonema* has been given. Phagotrophic feeding has been demonstrated in all. The descriptions include structural details visible only with the electron microscope as well as observations on behaviour and life-history visible only in the living material. The reasons for the use of the generic name *Chrysochromulina* Lackey are discussed in a preliminary way.

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STUDIES ON PIGMENT-ACTIVATING SUBSTANCES IN ANIMALS

I. THE SEPARATION BY PAPER ELECTROPHORESIS OF CHROMACTIVATING SUBSTANCES IN ARTHROPODS

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(Plates I and II in colour and Text-figs. 1–6)

Introduction

It has been established that many substances can bring about a change in position of pigments in crustacean chromatophores (Florey, 1951). The extracts of certain animal tissues (pituitary, crustacean sinus-gland and post-commissure organs, insect brain and corpora cardiaca) are especially active (Brown, 1940; Knowles, 1953; Dupont-Raabe, 1952; Thomsen, 1943); there is evidence that these tissues intervene in the normal colour change of the animals which possess them and that their products may properly be considered as hormones. On the other hand, many species which do not themselves possess chromatophores (oligochaetes, molluscs, and many insects) have nevertheless been shown to contain substances in their tissues which will, after injection into crustaceans, initiate pigment movements (Scharrer, 1954). It has not yet been ascertained whether these pigment-activating substances chemically resemble normal colour-change hormones or whether the pigment movements they produce are pharmacodynamic effects irrelevant in the study of colour physiology.

Many workers have made reciprocal-injection experiments in their studies of colour-change, and have found that for the most part the pigment-activating hormones are not species-specific, a fact which might seem to indicate some degree of chemical resemblance between chromactivating substances, though a number of observations have indicated that a chemical identity is unlikely (Brown, 1944). The interpretation, however, of the early reciprocal-injection experiments is complicated by the fact that many workers have referred to pigment changes after the injection of extracts without specifying with sufficient precision which pigments responded. The colour patterns of crustaceans are very complex, and it is now recognized that chromatophores with apparently identical pigments may differ physiologically in relation to their position on the body (Brown & Wulff, 1941; Knowles, 1955) and that, for instance, an injection may be followed by the concentration of some red pigments but by the dispersal of others. Injection and extirpation experiments have given rise to the suspicion that in a single crustacean species there may be a number of distinct chromactivating substances, each playing a specific role in the changing colour pattern of the individual. The probability of many colour-change hormones in a single individual makes comparison by reciprocal injection experiments difficult to assess, and it is clear that the ultimate resolution of the problem of the number and the identity of the various pigment-activating substances which have been extracted from animal tissues must await chemical separation and purification.

Chemical studies on the pigment-activating substances of crustaceans have hitherto been restrained by the difficulty of obtaining adequate material for analysis. For this reason the attempts by Abramowitz (1940) to purify the crustacean eye-stalk extract did not yield conclusive results (Brown, 1944). The techniques of paper chromatography and of paper electrophoresis overcome to some extent the problem of the separation of minute quantities of substances for study, and we have therefore used these methods in the present studies on pigment-activating substances. In this preliminary paper we give an account of experiments designed to provide at least a partial solution to two fundamental problems in the endocrinology of colour-change in arthropods, namely (1) whether there are chemical similarities between the pigment-activating substances which have been extracted from the sinus-glands and the post-commissure organs of crustaceans and from the brain and the corpora cardiaca of insects, and (2) how many pigment-activating substances are present in a single crustacean or insect species.

MATERIALS AND METHODS

Animals

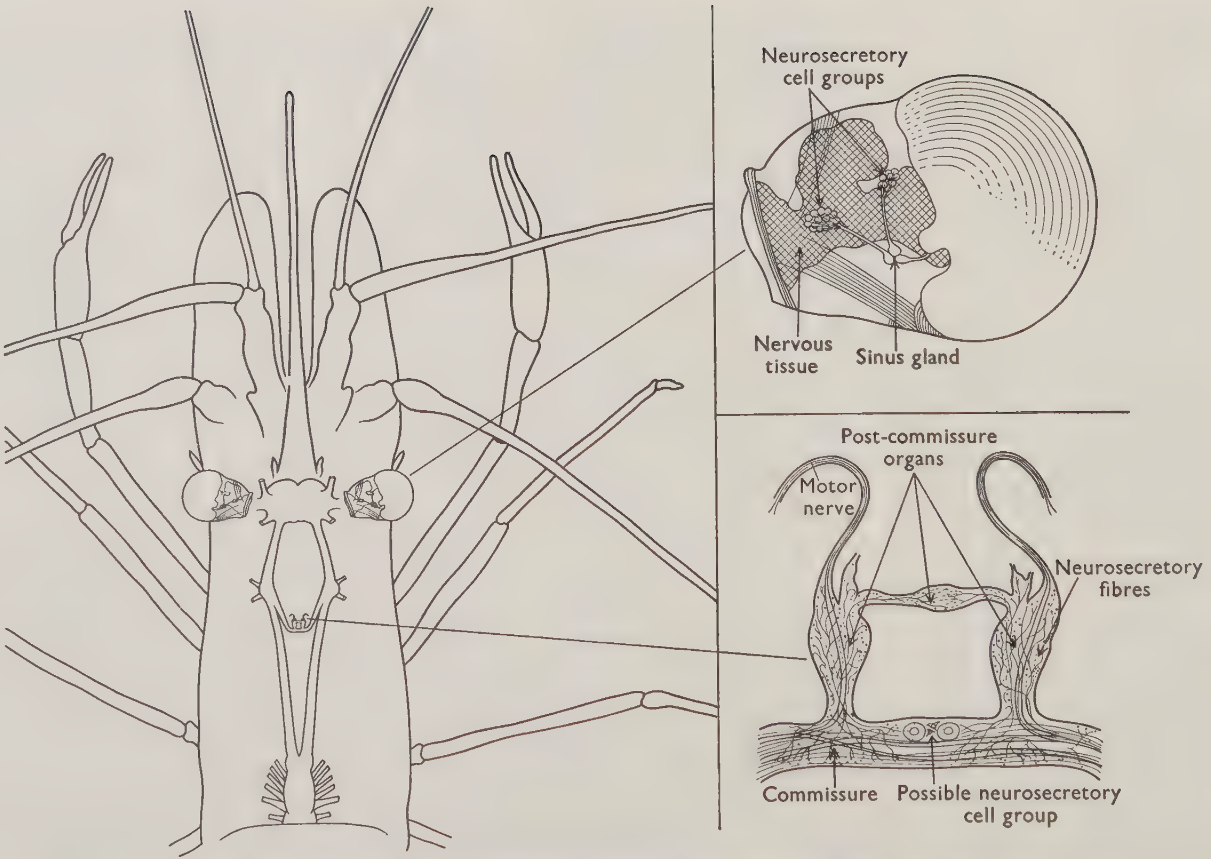
Three species of animals were used for testing the chromactivating potency of extracts, the crustaceans *Leander serratus* and *Crangon vulgaris* and the stick insect *Carausius morosus*, although others were used in some of the experiments as sources of extracts. *Leander* and *Crangon* were taken from various habitats near Plymouth and Roscoff, and stored until required for use in the aquarium tanks in running sea-water. *Carausius* were taken from the colony maintained by one of us (M.D.-R.) at the Sorbonne, and from that in the insect house of the Zoological Society of London.

Leander and *Crangon* were prepared as test subjects for this work by eye-stalk ablation. One eye of a male or non-ovigerous female was removed at the base by cutting through the arthrodial membrane with sharp-pointed scissors;

no precautions of sterility or of cauterization were taken. Twenty-four hours later the other eyestalk was removed in the same way. Animals were used after a further 24 h had elapsed or at any time later. Individuals which matched each other perfectly in colour were chosen for each experiment. Only those with dispersed pigments were selected. Single individuals were sometimes used for a number of tests, but never for more than one test on any one day.

The brain of *Carausius* was removed to prepare this animal as a test subject. The animal was anaesthetized with ether and the operation performed under Ringer's solution. The dorsal chitinous shield of the head was removed and the brain dissected out. The chitinous shield was replaced, to be held by clotting blood, and the animal allowed to recover. *Carausius* in the sixth or seventh instar were used, 24 h or more after the operation. After the removal of the brain *Carausius* takes on a pale grey colour as a result of the migration of the hypodermal melanin granules to the innermost depths of the cells, thus leaving less pigment near the surface. The result of an injection of chromactivating material is to provoke the migration of the melanin towards the surface so that the animals become darker in colour. A subjective scale of degree of darkening assessed macroscopically on the animal has been used throughout in determining the activity of injected extracts. Injections were made of about 0·05 ml. saline through a glass hypodermic needle passed in a forward direction through the arthrodial membrane at the base of one of the legs.

Colour change in *Leander*, as in most Crustacea, is a result of the migration of pigment in hypodermal chromatophores. There are several main types of these, and apparently similar ones on different parts of the body may behave in quite dissimilar manners. After eyestalk ablation the pigments of most chromatophores disperse, but not maximally; the injection of chromactive extracts produced either a greater dispersal or a concentration. In assessing colour change in *Leander* we have used a modified scale of the accepted chromatophore index of Hogben & Slome (1931), in which 1 represents full concentration and 5 full normal expansion of pigments; we have found, however, that under certain conditions some chromatophores expand some way beyond the normal state to a condition which we have called 5+, in which adjacent chromatophores appear, superficially at least, to anastomose (see Knowles, 1955, fig. 12). Using this scale we have in each experiment endeavoured to assess under the microscope the degree of expansion of the various types of chromatophores in different regions of the body. Our observations were made on as many as nineteen different chromatophore types, but only a few of these will be considered in any detail in this paper. Assessments were made 15, 30, 60 and sometimes 120 min or more after injection. Injections were made through a no. 28 steel hypodermic needle passed forward through the arthrodial membrane between the terga of the third and


Neurosecretory cell groups
Nervous tissue
Sinus gland
Post-commissure organs
Motor nerve
Neurosecretory fibres
Commissure
Possible neurosecretory cell group

Text-fig. 1. Diagram illustrating the approximate positions of the sinus glands and post-commissure organs of *Leander serratus*.

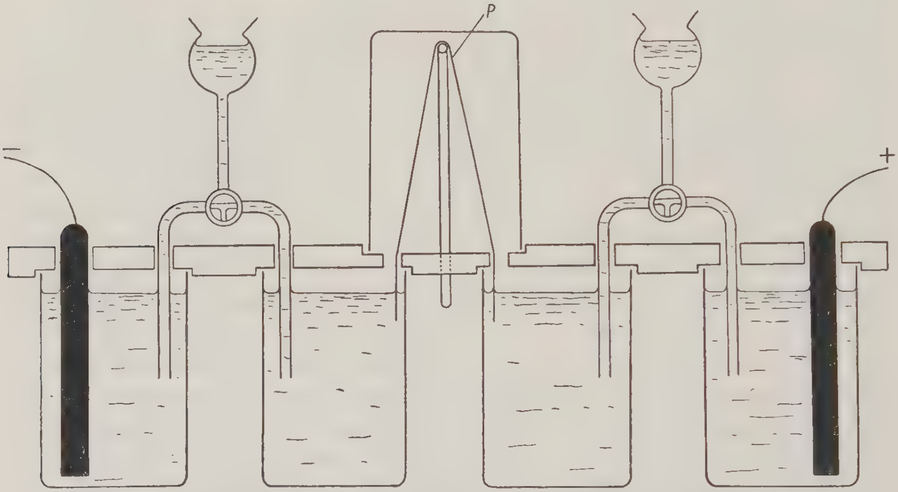
fourth abdominal segments, slightly to one side of the mid-line; the needle thus entered through muscle and was passed forward, directed towards the mid-line and slightly dorsally, till the point came to lie in the pericardium. 0·05 ml., or a little more, was injected each time into the main blood stream in this way, and the lateral muscle blocks served, by their contraction, to seal the hole, and so to reduce bleeding.

Preparation of Extracts

Four different organs were extracted in these experiments; they were the brain and the corpora cardiaca of insects and the sinus gland and post-commissure organs of crustaceans. The brain, and also the corpora cardiaca, which lie near it were removed from insects without anaesthesia and from animals which were intact, never from eyestalkless animals. Unless otherwise stated, all experiments were performed with males or non-ovigerous females. Each dissection of whatever kind took 4–10 min. The subsequent treatment of the organs for the various experiments varied greatly, but three main categories may be noted. In some of the earlier experiments the organs were crushed with forceps direct on to the central spot of the dry paper strip which was to be used for electrophoresis (*v. i.*). In other experiments the organs which were to be used in one electrophoretic separation were accumulated in a drop of distilled water until all dissections were completed. They were then ground up in a small mortar and the extract transferred to the point of origin on the paper strip, drop by drop, drying with hot air between each application. In all the later experiments the strip was first wetted with buffer solution, extractions were made in the same solution and applied to the wet paper immediately before electrophoresis; the extracts were not allowed to become dry at any time. All three methods were used in experiments on each of the four organs.

Electrophoresis

Electrophoresis was carried out upon filter-paper in two kinds of apparatus. In the earlier experiments at Plymouth the paper strip, 3 cm wide, was draped over a horizontal glass support and hung down on either side into the buffer vessels (Text-fig. 2). In the apparatus used at Roscoff the paper strips, several of which could be used simultaneously, were stretched horizontally. A more fundamental difference was that in the power pack used at Plymouth the current was stabilized, but the voltage allowed to fluctuate somewhat, while in that at Roscoff the voltage was stabilized but the current rose sometimes as much as 100% during the course of the experiment. Since it is not possible to stabilize both at the same time, it appears of more theoretical importance to stabilize the voltage, but in practice stabilization of either leads to adequate uniformity of results. In all runs phosphate buffer was used, N/15 or N/30. Unless otherwise stated, the pH was 7·5, each run at Plymouth lasted 16 h (less at Roscoff, but variable) and the length of paper was 28 cm. The extract which was to be separated by electrophoresis was applied to the central point of the paper, midway between the electrodes. In some of the later experiments a spot of LD-leucine was placed alongside to act as a marker; this was stained with ninhydrin at the end of the run and gave some measure of the constancy of conditions from one run to the next. Whatman's paper no. 1 was used.



Text-fig. 2. A diagrammatic section through the electrophoresis apparatus used at Plymouth; *p*, the filter-paper strip.

After electrophoresis the paper was cut into sections, usually after preliminary drying with hot air. In the earlier experiments the band extending 0·5 cm on either side of the origin was cut out and numbered 0. The remaining portions of the paper were cut transversely into five equal parts, 2·7 cm wide, on each side of the origin, numbered −1 to −5 at the cathode and +1 to +5 at the anode. The arrangement of the pieces was thus:

anode +5 +4 +3 +2 +1 0 −1 −2 −3 −4 −5 cathode.

In some experiments the region numbered −1, as it proved of exceptional interest, was further subdivided into three pieces, each 0·9 cm wide, numbered $-\frac{1}{3}$, $-\frac{2}{3}$, and −1. In the experiments performed at Roscoff, bands 1 cm wide on each side of the origin were cut out separately and numbered +0 and −0; the other bands, each 2 cm wide, started correspondingly farther out from the centre than in the Plymouth experiments. These various bands were eluted with 0·2 ml. of an appropriate saline or sometimes distilled water for a period of 15–30 min. The eluates were then injected, as described above, and the results recorded.

Further details of special methods used are mentioned in the experimental sections.

THE POST-COMMISSURE ORGANS

The effect of an injection of a post-commissure extract into an eyeless prawn differs significantly from the effects produced by the injection of a sinus-gland extract. A sinus-gland extract concentrates the small red and the large red chromatophores of the body and of the tail and is generally without effect on the white pigments (sometimes a slight concentration is observed after injection). During the first half hour after injection the effects on the large red chromatophores and on the small red chromatophores are indistinguishable, maximum contraction being produced in both type of chromatophore, but later the large red chromatophores begin to re-expand before any change in the small red chromatophores can be observed. On the other hand, injection of a post-commissure extract is followed by a contraction of all chromatophore pigments (including the white pigments), which lasts for 5–10 min., but thereafter the small red chromatophores of the body and certain red chromatophores of the tail begin to expand and in due course become more expanded than they had been before the injection. The large red and the white chromatophores, however, remain contracted for an hour or longer. Brown & Klotz (1947) reported that they could extract two substances from the commissure of *Crago* (=*Crangon*) by making use of differential solubility in alcohol. One of these (which they called CBLH) concentrated dark pigments in the body and tail of *Crago*, and the other (CDH) in the absence of the first, darkened the animal by promoting expansion of the dark chromatophores of the body and of the tail. We have by electrophoresis separated these two substances and have shown that there are also other chromatophorotropically active substances in the post-commissure organs.

The electrophoresis of post-commissure organ extracts was carried out at Plymouth and at Roscoff. In the Plymouth experiments care was taken to ensure that the current did not fluctuate during an experiment, but stabilization of the voltage was not complete. A relatively low current, ranging from 0·2 mA to 1 mA/cm width of paper was applied in this first series of experiments. In a later series of experiments, carried out at Roscoff, higher voltages, higher current strengths and shorter times were employed than in the first series and the apparatus used delivered a stabilized voltage, but the current increased during the course of an experiment. Each extract of the post-commissure organs was generally prepared from that portion of the post-commissure complex posterior to the commissure, namely the two post-commissure nerves and their lamellae (Knowles, 1953); in none of the later experiments did the extract contain any commissure material. The post-commissure organs used were dissected from large male individuals or non-ovigerous females, measuring 50–55 mm in length.

Plymouth Experiments

A series of experiments was carried out at Plymouth during January and July–August, 1954. In the preliminary experiments some of the commissure material was included with the post-commissure organs when the extracts were made. Each extract contained ten post-commissure organs and the extract was allowed to stand at room temperature while the organs were being dissected, an operation which generally took up to an hour to complete. The extract was then applied to the centre of a strip of Whatman no. 1 paper and dried under an electric hair-drier; after this operation the paper strip was subjected to electrophoresis, sometimes immediately, but usually a period of about $\frac{1}{2}$ h was allowed for equilibration before the current was switched on. In later experiments at Plymouth the organs from five individuals were used each time and the extracts were applied immediately to the paper: they were not allowed to stand at room temperature.

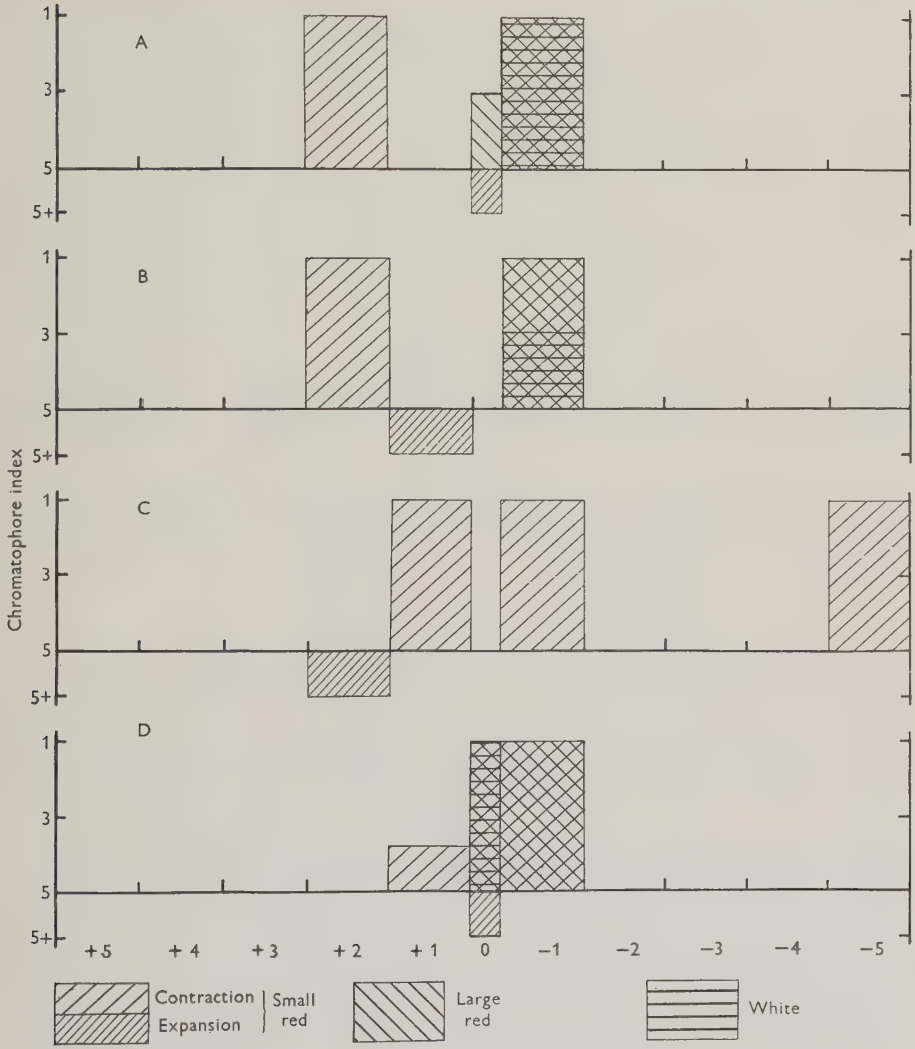
After electrophoresis the strip was dried once more under an electric hair-drier and then cut into portions each 2·7 cm long, with the exception of the central portion, containing the point of application of the extract, which was only 1 cm long. Each portion was then eluted separately in distilled water and the resultant solutions were injected into prawns from which the eyes and their stalks had been removed a week previously. The resultant effects are shown graphically at Text-fig. 3, in which the generally accepted chromatophore index has been modified slightly to give a means of expressing an expansion of pigments greater than that normally found in eyeless individuals. This extraordinary expansion is referred to as 5+.

Immediate Application (Text-fig. 3A)

In three experiments the organs were macerated first in water and then in methanol. Aqueous and alcoholic portions were applied separately to point of origin immediately after removal from the body and the resultant extract was applied within a few minutes to the paper which was then dried. It was hoped in this way to reduce to a minimum the possibility of chemical change of the extract before electrophoresis began. Electrophoresis was carried out at pH 7·5, 320–350 V, current 0·3 mA/cm, applied for 16 h.

In each of these experiments three portions of the paper yielded active extracts after electrophoresis (Text-fig. 3A), namely +2, zero and −1, and the effects of these extracts were different.

An injection of the −1 extract was followed by a contraction of all the chromatophores of the body and of the tail, namely the large red, the small red and the white chromatophores. The effects of this extract resembled those



Text-fig. 3. Diagrams of the results of typical experiments with injection of post-commissure extract into *Leander*, after electrophoresis. In each graph the horizontal axis represents the length of the paper strip, cut into numbered portions (see section on methods), and the vertical axis the chromatophore index after 60 min, in the prawns which were injected with the eluates of the corresponding fragment of paper. Only striking departures from the initial chromatophore index at the beginning of the experiment are recorded; minor variations are omitted. A, after immediate extraction, pH 7·5, 18° C, 16 h, 320–350 V, 0·3 mA/cm; B, organs left 1 h in water at room temperature before extracting, pH 7·5, 18° C, 16 h, 230–250 V, 0·2 mA/cm; C, organs left 4 h in water at room temperature before extraction, pH 7·5, 18° C, 16 h, 430–460 V, 0·5 mA/cm; D, extract boiled, pH 7·5, 18° C, 12 h, 230–250 V, 0·2 mA/cm.

produced by the −1 extract yielded by sinus gland and corpus cardiacum extracts but differed from them by reason of the presence of the white-pigment activator. The substance at −1, present in the three organs enumerated, we have called the A-substance (Carlisle, Dupont-Raabe & Knowles, 1955).

An injection of the zero extract was followed by a contraction of the large red chromatophores of the body and of the tail and of the white chromatophores of the body but by an expansion of the small red chromatophores of the body and the tail, especially those of the uropods. The general effect of this injection was to produce an individual with a dark body and with a scarlet tail; during the first 10 min after injection the bands of colour on the body turned blue as the large red chromatophores contracted sharply and a blue pigment diffused from them into the surrounding tissues; later this blue pigment disappeared and the bands of colour were hardly apparent. A maximally dark prawn with minimal display of pattern had been produced. We have called the material responsible for this effect the B-substance.

An injection of the +2 extract was followed by the contraction of the small red chromatophores only; the large red chromatophores remained dispersed and the white chromatophores were not affected. We call such a substance with a single effect an α-substance.

Delayed Application Experiments (Text-fig. 3B, C)

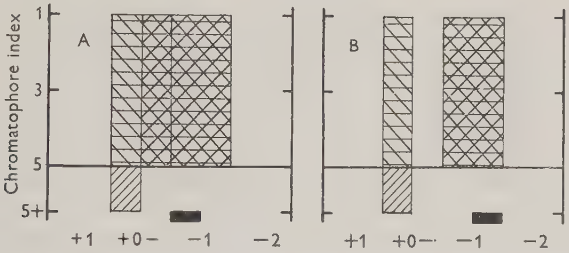
In four experiments the extracts were allowed to stand for an hour or more at room temperature before application to the paper. In one experiment the extract was left on the damp paper for 6 h before electrophoresis began.

The pattern of activity differed from that previously described in several ways. (1) The white pigment concentrator gradually disappeared from its position at −1, and after 3 h was no longer present in a detectable concentration. Sandeen (1950) has already reported that a white pigment-concentrating substance slowly disappeared from an extract of the tritocerebral connectives of *Uca*. (2) The pigment-dispersing substance becomes more mobile and moves towards the anode as far as 4 cm or more from the point of application of the extract. The effect of large red chromatophore concentration previously associated with the pigment-dispersing substance was no longer present, and one may speculate whether the large red chromatophore concentrating substance was originally linked to the pigment dispersing substance, and that the rupture of this link rendered the dispersing substance more mobile. If, this is so, the B-substance contains two components active on red pigments; one ('B_X') disperses red pigments; the other ('B_C') concentrates the red pigments of the large red chromatophores. (3) The −5 portion of the paper yielded an extract which concentrated the small red chromatophores. This additional α-substance had not been detected in the immediate extraction experiments and it seems likely to be a disintegration product of some larger molecule.

Roscoff Experiments

The first series of experiments having shown that when fresh extracts were used the greater part of the substances with effect on the chromatophores were relatively immobile under the conditions used in the experiments, a further series of experiments were undertaken with the express intention of separating those relatively immobile substances. A higher voltage and current were used but for a shorter time; and the zero portion of the paper was cut into two portions, one on each side of the centre, in order that any slight migration might be detected.

In the second series of experiments the extracts, which were made in buffer solution, were not allowed to become dry at any time; they were applied to *damp* paper, which was then immediately subjected to electrophoresis. A spot of DL-leucine was added at the edge of the paper in these experiments to serve as a marker.



Text-fig. 4. As Text-fig. 3, but showing two experiments performed at Roscoff. The black rectangle represents the position taken up by a spot of DL-leucine. pH 7·8, 18·5° C, 5 h, 320 V, 0·8 mA/cm.

After electrophoresis the still damp paper was cut into portions and extracted at once with sea water, while a thin strip along the edge of the paper was dried and stained with ninhydrin to detect the leucine. The immediate application method was used in each experiment.

The most significant feature of these later experiments was the complete separation of the A- and the B-substances (Text-fig. 4), and the demonstration that the B substance bears a slight negative charge, even when both B_X and B_C effects are produced. Another interesting feature of these later experiments was the distribution of the white-pigment-concentrating substance. At 320 V and 0·8 mA/cm it did not apparently move far from the point of application and sometimes not so far as did the A-substance; its distribution in this case strengthens the suspicion that it is not normally bound to the A-substance. If it is bound to any other substance, it would seem more probable that it may be bound to the B-substance, for in a boiled extract it is found associated with the B- rather than with the A-substance (Text-fig. 3D). This suggestion would also help to explain the otherwise somewhat baffling distribution of the white-pigment-concentrating substances in the experiment illustrated in Text-fig. 4A. The distribution becomes more reasonable if we suppose that some of the white-pigment-concentrating substance has become detached from the B molecule but that some still remains, but we have not yet sufficient evidence to establish that the white-pigment-concentrating substance forms part of a larger molecule.

Environmental and Sex Differences

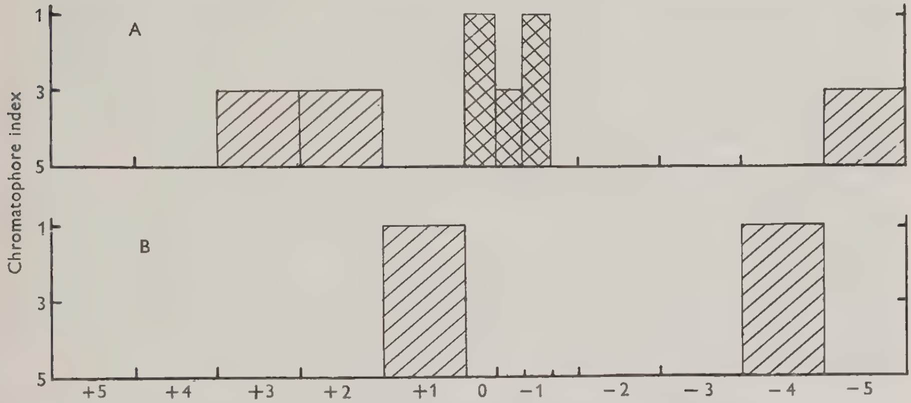
Most of the experiments have been carried out using extracts prepared from male individuals or from a mixture of males and non-ovigerous females, but we have sometimes perforce used females only. The prawns have generally been gathered close inshore in water 1–6 m in depth, but once prawns were used which had come from greater depths—40 or more metres. We have not yet sufficient data to dogmatize, and it is hoped that an investigation of environmental and sex differences, if indeed they do exist, may form the subject of a later publication, but it is worth putting on record here that the electrophoretic patterns which we have so far described may only be typical of shallow-water, male animals. As we have shown, these seem to contain a mixture of A- and B-substances, but when only females were used in the preparation of the extracts little or none of the B-substance was found. On the other hand, male prawns from greater depths seemed to contain an abundance of the B-substance, but little or none of the A-substance. These deeper-water prawns were noticed to be different from those gathered in shallower waters, being almost scarlet in colour and with maximal expansion of the small red chromatophores.

THE SINUS GLAND

The general pattern of these experiments resembled that of the experiments already described. Fresh and stored extracts were used from prawns of both sexes gathered from various localities in the Plymouth area. Normally ten glands were used for each extraction, but in later extractions the number was sometimes smaller.

Immediate Application Experiments (Text-fig. 5A)

In these experiments the glands were dissected out under isotonic saline, immediately crushed on to the spot of origin on the filter-paper strip and dried with a blast of hot air. Each dissection took 4–7 min. As soon as all the glands had been applied in this manner the paper strip was placed in the electrophoresis apparatus and, after equilibration, the current was switched on. In such a run the distribution of chromactive substances was similar to that found with extracts of the post-commissure organs, except that no consistent effect was observed on the white chromatophores, nor was any sign apparent of any substance tending to provoke expansion of the small red chromatophores (Text-fig. 5A). About the middle of the −1 band (which was frequently subdivided for elution into smaller sections) was a substance provoking strong concentration of all red pigments, but with a rather stronger action on the small red than on the large red chromatophores. At zero a substance effective in concentrating red pigments was sometimes found, but we have not sufficient evidence that this is a distinct substance. At about +2 and −5 were found small quantities of more labile substances—the α-substances—acting only on



Text-fig. 5. As Text-fig. 3, but extracts of sinus glands. Band −1 was subdivided into three. A, after immediate extraction; extract derived from large, blue, non-ovigerous females, pH 7·5, 18° C, 16 h, 320–350 V, 0·3 mA/cm; B, organs left for 12 h in water before extraction, pH 7·5, 18° C, 12 h, 320–350 V, 0·3 mA/cm.

the small red chromatophores. These α-substances appeared similar in all respects to those of the post-commissure organ extracts.

In a small number of experiments a slight concentration of the pigment of the white chromatophores was found after injection of material from about −3. This may be an artifact, for the white chromatophores are directly light-sensitive and may be affected by slight alterations in the intensity of illumination (Knowles, 1940). We have, however, sometimes observed a similar effect after injection of whole fresh extracts of sinus gland (see also Knowles, 1939).

Delayed Application Experiments (Text-fig. 5 B)

For these experiments the first gland dissected out was placed in 0·1 ml. distilled water, and each succeeding gland added to this. When all the dissections were completed the material was ground in a small mortar, with addition of a further 0·1 ml. water, and the extract applied, after a variable interval of time, to the dry paper by successive applications with a capillary pipette. Each successive application was dried with a blast of hot air. The residue, after the aqueous extract had been transferred to the paper, was extracted further with methanol, which was then applied to the same spot on the paper.

When an extract was kept for some hours at room temperature before application to the paper the electrophoretic pattern obtained was quite different from that found with fresh extracts (Text-fig. 5 B). Little or no chromactivating substance was found at zero or −1 under these conditions, but an abundance of the α-substances was found at −4 or −5 and at +1 or +2, with a strong concentrating effect upon the small red chromatophores.

Corpora cardiaca and Brain of Insects

For the comparative study of the chromactivating substances of the corpora cardiaca and brain of insects we have chosen a species, *Carausius morosus*, which itself shows colour changes. The active substances in the extracts of these organs, after separation by electrophoresis, were tested on the living insect and on the crustaceans *Leander serratus* and *Crangon vulgaris*.

Whole extracts of the brain and of the corpora cardiaca of insects are each active upon the pigments but their effects differ, both upon insects and upon Crustacea. Whole extracts of corpora cardiaca provoke a strong concentration of the red chromatophores of *Leander* (Thomsen, 1943; Brown & Meglitsch, 1940) and the black of *Crangon* and *Ligia* (Dupont-Raabe, 1952), but their activity is only moderate upon the pigments of *Carausius*: after injection of a total extract of corpora cardiaca the animals do not take on the black colour characteristic of complete darkening, but pass only from light grey to dark grey. Total extracts of the brain, on the other hand, are completely inactive on the pigments of *Leander*, but act very strongly on the pigments of insects and possess some degree of activity on the black pigments of crustaceans such as *Crangon* and *Ligia* (Dupont-Raabe, 1949, 1951, 1952).

The conditions of electrophoresis were as described above. Ten organs were usually used for each separation. Dissections were made rapidly under Ringer's solution ('Rabbit Ringer'); the organs immediately after injection were placed in a small quantity of distilled water, ground up and transferred directly, or sometimes after boiling, to the paper strip.

Corpora cardiaca

Injection of eluates, of different portions of the paper strip after electrophoresis, into *Leander* revealed the existence of a band at -1 which was very strongly active upon the small and large red chromatophores. It provoked a strong contraction of all the red chromatophores of the body and tail, but had no effect on the white pigments. In its effects and in its electrophoresis migration it seems comparable to substance A present in extracts of the post-commissure organs and sinus glands of *Leander*. At pH 7·5 eluates of the part of the paper near the origin and 1 cm towards the cathode (0 and $-\frac{1}{3}$) were often rather strongly active, especially upon the small red chromatophores. At pH 5·0 the migration of this substance towards the cathode was much stronger: it was not present in portions 0 and $-\frac{1}{3}$, but revealed its presence at -1 and -2.

Besides substance A, another substance is evidently present in extracts of corpora cardiaca—a more mobile substance which provokes concentration of only the small red chromatophores. After electrophoresis at pH 7·5 for 16 h at 0·3 mA/cm, 320–350 V the band corresponding to this substance was to be found at $+2$ and $+3$. With a higher current and voltage (1 mA/cm, 540–580 V) it migrated to $+5$. This substance may correspond to the α-substance found at $+2$ in sinus gland and post-commissure organ extracts, representing a disintegration product of the A-substance. Such an hypothesis is supported by the fact that this α-substance cannot be detected if electrophoresis is performed with a boiled extract (in which presumably the enzymes are destroyed), which, however, retains the A-substance, at -1 in full strength.

Substance A is responsible for the action of corpora cardiaca extracts upon the melanophores of *Crangon*. In a series of experiments in which the eluates of the central portions of the paper strip were injected simultaneously into *Leander* and *Crangon* the responses obtained, though different in kind—concentration of the red pigments in *Leander* and of the black in *Crangon*—were always coincident.

The identification of that substance of the corpora cardiaca which is active upon the pigments of insects offers some difficulties, and the results are not yet clear. It will be recalled that total extracts of corpora cardiaca provoke only a moderate darkening of insects, and after electrophoresis, with its attendant losses of activity, the concentration in the eluates is lower and the reactions obtained still weaker, so that the delimitation of the zones of activity is difficult. The positions of the active zones, so far as they have been determined, sometimes coincide with substance A and substance α, but sometimes we have obtained no response at -1, while positive responses have been observed with the eluates of portions of the paper inactive upon crustacean pigments, notably $+1$ and -4. Thus it seems improbable that it is the A- and α-substances in total extracts of corpora cardiaca which influence the insect pigments, the more so as in the only two exploratory experiments we have performed with sinus gland extracts we have not found an exact correspondence in position of substances active upon insects and Crustacea. In later experiments, performed at Roscoff, with a higher voltage and an apparatus permitting several simultaneous runs, a detailed comparison was made, in the central region of the paper from $+2$ to -2, between the positions of substances active upon insects from extracts of the corpora cardiaca and brain. These experiments showed clearly that the tritocerebral chromactivating substance of the brain (substance C) which was so strongly active upon the pigments of the insect, was not present in extracts of corpora cardiaca.

The Brain

Electrophoresis of extracts of the brain at pH 7·5 revealed the existence of a substance of very low mobility, which was to be found on both sides of the origin ($+0$ and -0). This substance brought about a maximal darkening of the insect, analogous to that obtained with total extracts, and also a concentration of the melanophores of *Crangon*, an effect which was, however, not like that of substance A of the corpora cardiaca. It is totally inactive on pigments of *Leander*. The position of this substance on the paper did not coincide with either substance A or substance B and we have called it the C-substance.

It appears probable that, as with substance A, the C-substance may decompose into more mobile substances of lower molecular weight; parts of the paper situated rather far from the point of application of the extract often showed quite strong activity upon *Carausius*, particularly $+3$, $+4$ and $+5$. We may call this tentatively the γ-substance(s). It will be interesting in the future to compare in detail these substances with the mobile α-substances of the corpora cardiaca which are active upon insects and to establish if they are similar or perhaps identical.

No portion of the paper provoked concentration of the red pigments of *Leander*: substance A does not exist in the brain. Injection of total extracts had revealed no activity, but this might have been due to the existence of masking by an antagonistic substance; this possibility appears to be eliminated by the results of the electrophoresis experiments. Nor has any substance been found in the brain of insects which provokes expansion of any pigments of *Leander* or *Crangon*.

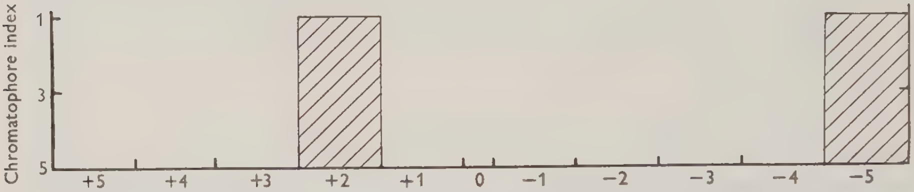
The insects thus possess at least two different chromactivating substances: substance A in the corpora cardiaca and substance C in the brain, localized in the tritocerebral region (Dupont-Raabe, 1954). It is difficult to pronounce any opinion on the origin of the A-substance, present in the corpora cardiaca, and upon its significance in the physiology of the insect, since its presence does not seem to be necessary for the normal colour changes. It does not seem to have its origin in the pars intercerebralis of the brain, where are situated the cells from which the internal cardiac nerves run; nor in the lateral protocerebral neurosecretory cells. Extracts of these parts dissected out from the brain, and of the internal cardiac nerves, are quite inactive, even after boiling, upon the pigments of *Leander* and those of the insect. Perhaps substance A is a transformation product of substance C, arriving at the corpora cardiaca by migration along a third pair of tritocerebral-cardiac nerves whose existence has only just been discovered (Dupont-Raabe, 1955). Yet a single experiment performed with a *Carausius* deprived of its brain 5 days previously has shown no diminution in the content of A-substance in the corpora cardiaca; this would suggest (though this is against the current of present day speculation) that the cells of the corpora cardiaca themselves may elaborate substance A.

It is possible that the substances of the brain and above all of the corpora cardiaca which bring about pigmentary movements in insects, intervene equally in the regulation of other physiological phenomena, such as heart-beat, water metabolism or the genesis of pigments—all phenomena whose regulation certainly involves the brain and corpora cardiaca (Wigglesworth, 1954; Cameron, 1953; Stutinsky, 1953; L'Hélias, 1955)—and that a chromactivating potentiality is possessed in various degrees at times (perhaps incidentally) by a precursor and by different products of disintegration.

Dialysis Experiments

In these experiments extracts were dialysed against distilled water or saline through a variety of semipermeable membranes. No significant variations were observed when the following membranes were used: British Cellophane Company's cellophanes PT 600, PT 400, PT 300; British Cellophane Company's wet cellulose film ref. no. 2/468/EHD/WR; two different samples of synthetic sausage skin. All these membranes allowed the passage of eosin, methylene blue (or at least some component of it), and a pink constituent of trypan blue, but failed to allow the passage of congo red. For experiments the extract selected was placed in a cavity slide and covered with a membrane; 0·25 ml. of distilled water or of saline was then placed on top of the membrane and the whole covered with an inverted Petri dish. Under these conditions an hour's dialysis sufficed for the passage of detectable quantities of chromactivating materials from the fresh whole extracts of sinus gland of *Leander* *serratus*, *Homarus vulgaris*, *Carcinus maenas*, *Maia squinado* and *Cancer pagurus* from fresh whole extracts of post-commissure organs of *Leander serratus* and of corpora cardiaca of *Carausius morosus*. When, however, extracts of the brain of *Carausius* were dialysed the dialysate was much less active than the residue; it produced its action more quickly after injection, but the action was ephemeral, disappearing after 2–3 h, while that of the residue persisted for at least 4 h.

It was evident in these preliminary experiments that not all the material in the extracts was able to pass through the membranes which we used, for the activity of the dialysate was frequently different in kind from that of the residue. This was especially noticeable with extracts of the post-commissure organs, where apparently the B-substance did not pass, for the dialysate had a concentrating effect on the red pigments while the residue had a strong dispersing effect (Pl. II, fig. 10).



Text-fig. 6. As Text-fig. 3, but extract derived by leaving intact post-commissure organs in saline for ½ h and then removing them from the saline without maceration; saline used without further treatment. Note absence of the A-substance. pH 7·5, 18° C, 16 h, 380–430 V, 0·4 mA/cm.

In order to determine which fractions were and were not able to pass the membranes dialysis was performed on the eluates of various portions of filter-paper strips after electrophoresis of the extracts. It was found that the A- and B-substances and the C-substance were incapable of passing the membranes (Pl. II, fig. 12). The more transient α-substances which are to be found at about +2 and −5 on the papers and the γ-substances readily passed the membranes. These results hold for extracts of sinus gland, post-commissure organ and corpora cardiaca and insect brain.

With the intention of using the cell membranes as the semi-permeable membrane for dialysis experiments, post-commissure organs were dissected out carefully with a portion of the commissures and connectives attached. These were allowed to stand in isotonic saline for half an hour and then the saline was subjected to electrophoresis. The resulting pattern (Text-fig. 6) lacked substances A and B, possessing only the more labile α-substances. In further experiments the dissection was performed as before and the preparation left to stand for five minutes in saline. When this saline was injected it was found to contain no detectable chromactivating substances. Fresh saline was then added to the preparation and the connectives stimulated with a square-wave stimulator (100 c.p.s., 1·5–2 V) for 2 min only. Some of the saline was then subjected to electrophoresis and some of it injected direct into *Leander*. This showed a strong effect in concentrating the red pigments. The electrophoretic pattern once more lacked the A- and B-substances; the α-substances, however, were present in abundance. It appears likely, therefore, that the chromactivity of the dialysates we have tested is due to the α-substances (and γ-substances) and that the A-, B-, and C-substances, by reason of the size of their molecules or the charges they bear, do not readily pass the membranes.

Discussion

It is evident that paper electrophoresis provides a precise means of dealing with the very small amounts of pigment-activating substances that are present in insect and crustacean organs. Our preliminary studies have shown that the method provides a simpler and cleaner separation than the method of differential solubility in alcohol adopted by Brown and his collaborators, while at the same time supporting their contention that a number of different chromactivating substances are present in crustaceans. Brown & Klotz (1947) showed that the alcohol-soluble fraction of the commissure, which they called CBLH, concentrated the body chromatophores of *Crago* but was without effect on the tail chromatophores; it seems probable that this may correspond to the 'A-substance' which we have found in the extracts of the post-commissure organs as well as in the sinus-gland and the corpora cardiaca. The alcohol-insoluble fraction of the commissure (CDH) darkened both the body and the tail of *Crago*; it seems likely that this is the B-substance that we have isolated from commissure organ extracts. Clearly therefore our paper-electrophoresis studies thus far corroborate the work of Brown & Klotz, but in addition they provide evidence that other pigment-activating substances besides the A- and the B-substance may be present in extracts of the sinus-gland and the post-commissure organs.

The early work on the 'eyestalk hormone' suggested that it was chemically a very stable substance. Abramowitz (1940) found that boiling, even in HCl, appeared to increase the activity of the extracts used. Perkins & Snook (1931) reported that desiccated eyestalks would retain their activity for long periods of time. Our electrophoresis studies suggest that this appearance of stability can be to some extent illusory. While it is true that sinus-gland and post-commissure organ extracts of *Leander* still retain pigment activating potency after some hours in solution at room temperature it is clear that the active substances present after some hours are not those which are present in the neurohaemal organs immediately after they are removed from the body. It has already been reported that the white-pigment activator disappears fairly rapidly from *Uca* commissure extracts which are allowed to stand at room temperature and that the black-dispersing factor disappears, though more slowly (Sandeen, 1950). Our experiments suggest, moreover, that a red-pigment concentrator (the A-substance) becomes chemically transformed if the extracts are allowed to stand at room temperature, although what appear to be the transformation products (the α-substances) still retain some red-pigment-activating potency. We did not find α-substances after electrophoresis of boiled extracts; this agrees with Sandeen's observation that brief boiling retarded the disappearance of activity from *Uca* extracts.

We have not yet determined whether the α-substances are normally produced and released into the blood of *Leander*; if in fact they are the true hormones and the A-substance is a precursor. The greater mobility of the α-substances and the fact that they pass more readily through a cellophane membrane than do the A- and B-substances supports the idea that they would, if present in the animal, pass freely into the blood-stream, but we cannot yet with certainty state that they are normal blood-borne substances; the evidence suggests that they are fragmentation products of the A- or B-substances, but it is possible that they may be artificially produced during the preparation of the extracts. The results of the experiments in which the connectives were stimulated electrically support the idea that these α-substances are indeed among the actual blood-borne hormones, while the A and B substances are not, but they offer no proof of this. It is certain, however, that immediate extraction of a fresh organ gives different results from a slightly more delayed extraction, and any consideration of earlier work on the chemistry of the eye-stalk extracts and deductions from injection experiments should be reviewed in the light of this knowledge. It is clear that any future studies on the chemical analysis of chromactivators must distinguish between true hormones and precursor substances. The mode of preparation of extracts would seem to be all-important in this respect.

It is interesting to find that the sinus-gland and the post-commissure organs of crustaceans possess a chromactivating substance that resembles physico-chemically a substance present in the corpora cardiaca of insects. Hanström (1940), Brown & Meglitsch (1940) and Thomsen (1943) showed that extracts of the corpora cardiaca concentrated the red pigments in crustacean chromatophores but they did not bring forward any chemical evidence to suggest that the corpora cardiaca substance resembled the sinus-gland substance. Indeed, Brown (1944) has suggested that the chromactivating content of the sinus-gland and the corpora cardiaca may differ. In his experiments in collaboration with C. M. Stuter (unpublished) he claimed that the results indicated that the sinus-glands contained a precursor of the pigment-activating hormone but that the corpora cardiaca did not. The position of the pigment-activating substances after electrophoresis of sinus-gland and corpora cardiaca extracts indicates that the A-substance in the insects and the crustaceans may be chemically similar though as yet we cannot prove identity. The significance of the A-substance in insects is not yet clear, unless, as our electrophoresis studies have suggested, it may itself be a precursor of other substances.

Our studies have shown that the behaviour of the brain pigment-activating substance of *Carausius* after electrophoresis differs from any pigment-activating substance that we have separated from the neurohaemal organs of crustaceans. It is also clear that this substance, which we have termed the C-substance to distinguish it from the crustacean chromactivating substances, is ineffective when injected into *Leander*. It does, however, bring about pigment-concentration in the chromatophores of *Ligia* and *Crangon* (Dupont-Raabe, 1952). We may therefore speculate whether the C-substance is absent from crustaceans, or whether it is a physiologically active colour-change hormone of some species. It is perhaps significant in this respect that *Crangon* and *Ligia* differ from *Leander* but resemble *Carausius* in the presence of melanin in their pigmentary effector systems.

It is interesting to find that the method of paper electrophoresis separates from crustacean organs more than one substance which concentrates red pigments in the chromatophores. This finding suggests that both precursor and active substances may be present in extracts of crustacean neurohaemal organs, and that the precursor substances too have some pigment-activating potency. Such a concept would provide a possible explanation for the presence of pigment-activating substances in organs of species which do not themselves possess chromatophores.

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SUMMARY

Pigment activating substances in the prawn *Leander serratus* and the stick insect *Carausius morosus* have been compared. Paper electrophoresis has been used successfully to separate substances in extracts. A substance which we have called substance A appears to be present in extracts of sinus glands and post-commissure organs of *Leander* and in extracts of corpora cardiaca of *Carausius*: it seems very similar or possibly even identical in all three types of extracts. It stimulates contraction of all the red pigments of the body of *Leander*. It is incapable of passing the semipermeable membranes which we

have used, and possesses a low mobility at pH 7·5. There is evidence suggesting that it is not the definitive hormone which is released into the blood, but a precursor which is split before release. What appear to be disintegration products of substance A are present in extracts and increase in amount at its expense if an extract is left standing. These substances—the α-substances—pass freely through a dialysis membrane and have a high mobility at pH 7·5. Only the α-substances are released under the effect of electrical stimulus of the commissure when the post-commissure organs are lying in a saline bath. The α-substances affect only the small red chromatophores of *Leander*.

Another substance of low mobility was found only in extracts of post-commissure organs. This substance B concentrated the pigment of the large red chromatophores only and expanded the pigment of the small red chromatophores of the body and tail. It is antagonistic to substance A. It is incapable of passing a dialysis membrane.

Substance C is present only in extracts of the brain of *Carausius*. This substance provoked a darkening of *Carausius* and concentration of the black pigments of *Crangon*, but was without effect on *Leander*. Substance C has low mobility and cannot pass a dialysis membrane.

At least two chromactivating substances are present in *Leander*, and two in *Carausius*. The mechanism of colour change appears to be totally different in the two species, but *Carausius* possesses one of the substances which is concerned in the colour change of *Leander*, and another of its chromactivating substances is active upon *Crangon*, which, unlike *Leander*, possesses melanophores. It may be that melanin is under the same control in both insects and crustaceans.

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EXPLANATION OF PLATES

Colour photographs of *Leander serratus*, taken on Kodachrome film (Figs. 1 and 2) or on Ferraniacolor film (Figs. 3–12) with electronic flash.

PLATE I

Fig. 1. A dorsal view of the cephalothorax of *Leander* illustrating some of the chromatophore types—the white reflecting chromatophores, the large red-yellow chromatophores forming the bands, and the small red-yellow chromatophores between.

Fig. 2. A group of expanded chromatophores (chromatophore index 5) from the cephalothorax; white, large and small red, and faintly under each red chromatophore its yellow component; note the red component of the white chromatophore.

Fig. 3. The edge of one of the uropods, showing all pigments moderately dispersed (chromatophore index 4–5).

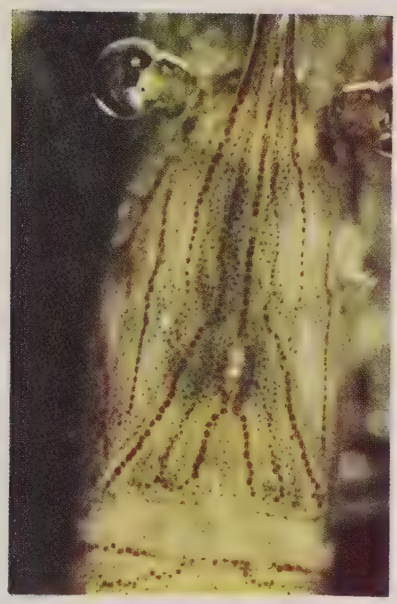
Fig. 4. Two large red chromatophores of one of the uropods, moderately contracted (chromatophore index 2).

Fig. 5. The same two chromatophores moderately expanded (chromatophore index 4–5).

Fig. 6. An eyestalkless *Leander serratus* which had been injected ½ h previously with 0·05 ml. sea water (control).

Fig. 7. A similar animal which had been injected with an extract of one sinus gland in 0·05 ml. sea water—concentration especially of small red chromatophores, thus enhancing the pattern.

Fig. 8. A similar animal which had been injected with an extract of one post-commissure organ in 0·05 ml. sea water—concentration especially of the large red chromatophores of the bands, thus obscuring the pattern.



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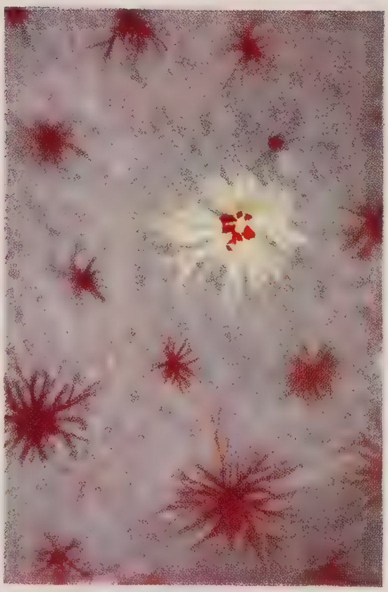

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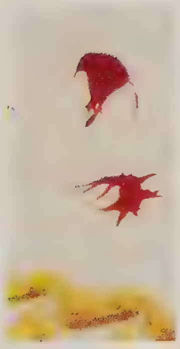

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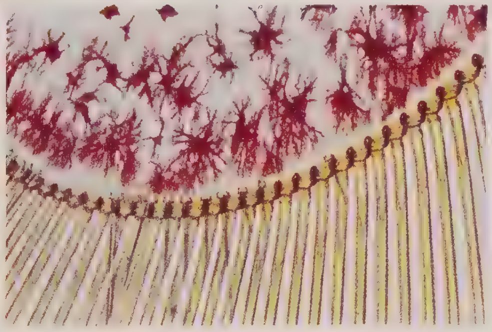

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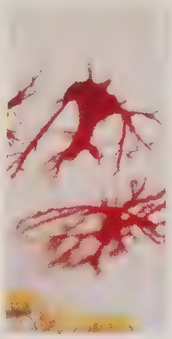

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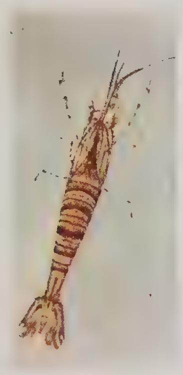

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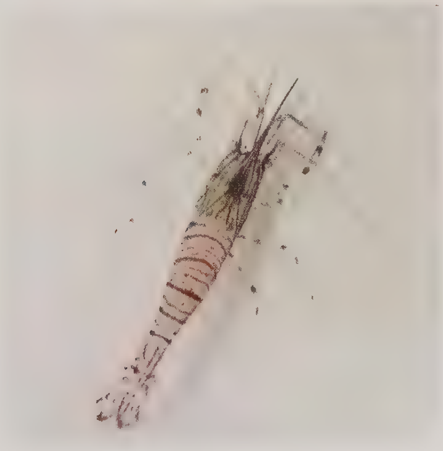

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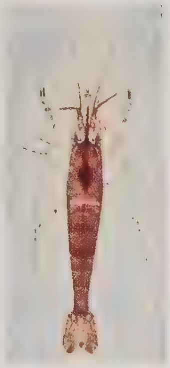

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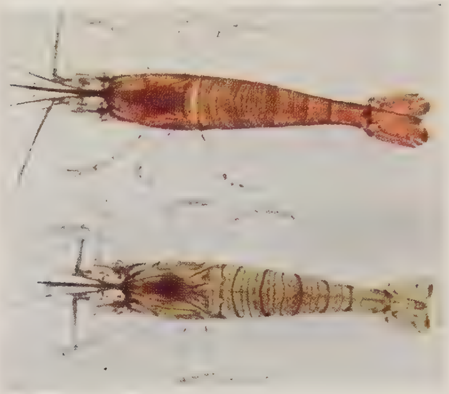

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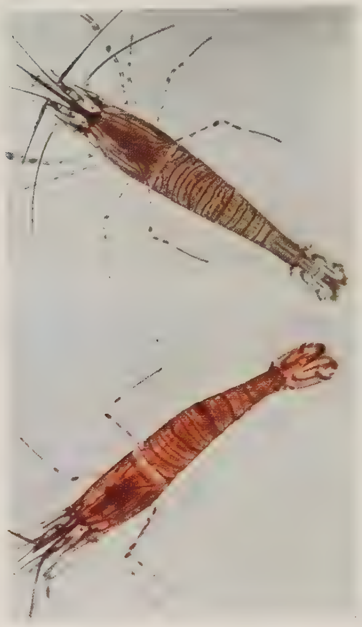

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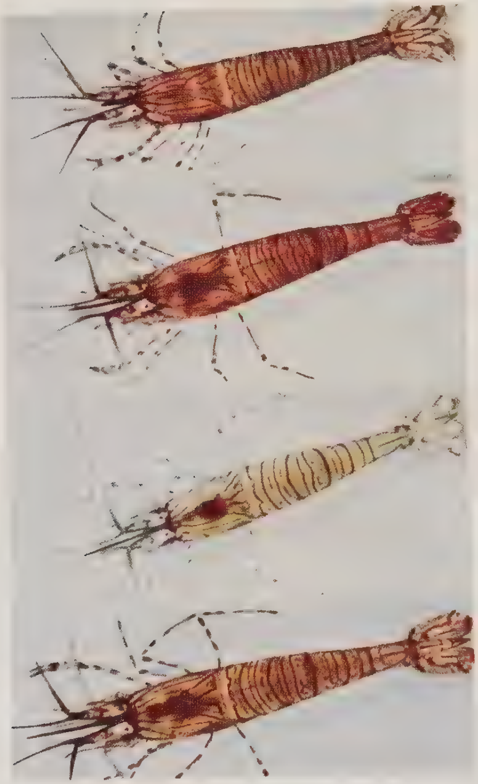

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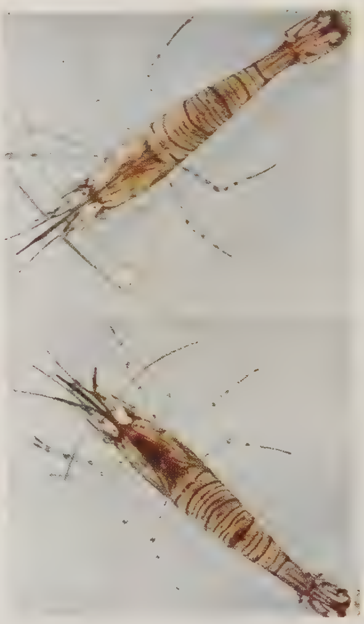

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PLATE II

Fig. 9. Electrophoretic separation of chromactivating substances from an extract of post-commissure organs. Under the conditions of this experiment substance B remains at 0, producing a hyperexpansion of the small red chromatophores to 5+ but a concentration of the large red chromatophores; substance A migrates to −1, concentrating all the red chromatophores. Photograph taken 30 min after injection.

Fig. 10. Separation of chromactivating substance by dialysis. Half an hour before this photograph was taken the left animal had received the fraction of a post-commissure organ extract that had passed through a cellophane membrane: this fraction contained α-substances; the animal on the right received the fraction that did not pass through the membrane: the effect is predominantly that of substance C.

Fig. 11. Separation of chromactivating substances by dialysis. The animal on the left received the fraction of a sinus gland extract which had passed through a cellophane membrane; the right-hand animal that which had not passed through.

Fig. 12. As Fig. 11, but using the eluate of the −1 fraction of the paper strip after electrophoresis of the sinus gland extract: the A-substance is incapable of passing the cellophane membrane.

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BLOOD PERFUSION OF THE KIDNEY OF *LOPHIUS PISCATORIUS* L.

IV. MAGNESIUM EXCRETION

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(Text-figs. 1–3)

From our previous publications (Brull & Nizet, 1953; Brull, Nizet & Verney, 1953; Brull & Cuypers, 1954), it appears that the power of concentration of the aglomerular kidney of *Lophius* is rather low, the density of the urine being usually less than that of the plasma concentration. Most individual urinary constituents do not reach concentrations that are more than once or twice those in the plasma, magnesium being an exception.

The kidney of *Lophius* possesses a high power for concentrating magnesium, to a degree approaching a hundred-fold. The plasma Mg usually increases after the fish has been caught, and therefore it seems likely that trauma is a factor in increased Mg absorption.[2] We referred in our previous work (1953) to other authors who had already noticed that urine secreted by *Lophius* in the aquarium is not the same as that secreted by fishes living under normal conditions. Yet, as *Lophius* kidneys are endowed with a particular power for excreting Mg, we thought it would be interesting to investigate this function further. What is the normal amount of Mg in *Lophius* plasma? Is it higher or similar to the figures found in mammals? Is there a threshold below which the secretion stops? Is exogenous Mg excreted in the same manner as the Mg that enters the blood stream through normal channels? These are the first questions which arise and seem worthy of investigation.

METHODS

The problem was studied with isolated kidneys perfused with heparinized blood, according to the method described by Brull & Cuypers (1954). As we demonstrated that there is a maximum or optimum perfusion pressure above which no further increase of urine secretion is obtained (250–300 mm water), our perfusions were made at pressures of about 250–300 mm of water, at room temperature and with oxygenated blood (in July–August). Leaving aside a first series of experiments, in which the initial plasma level of Mg was high, we shall describe briefly three experiments only in which, during prolonged perfusion of the kidney, total exhaustion of the plasma Mg was reached.

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[1] With the collaboration of A. Dujardin, L. Dubois and L. Wilsens.

[2] In dialysis experiments on *Lophius* skin E. Nizel (unpublished) found that rubbing the skin increases its permeability to minerals.

Determinations of Mg were made by the method of Fister (1950), with a modification described by M. Orange & H. C. Rhein (1951).

Some of the determinations were checked with the ammonium magnesium phosphate method, and the figures agreed. Trichloracetic deproteinization was used. When the plasma figures were low, 2 ml. of plasma were used instead of 0·5 ml. Urine samples were diluted.

Experimental Results

Figs. 1–3 illustrate three perfusion experiments in which, with the use of two kidneys, the plasma Mg could be exhausted in 3–5 h, Mg being added afterwards.

In Expt. 54B (Fig. 1) a perfusion was started with 300 ml. of blood with a cell volume of 16%, and an initial plasma Mg of 16·2 mg. Two kidneys, weighing 12·5 and 12 g respectively, were perfused. As usual, there was an initial rise in urinary Mg, probably due to the fact that the kidneys came from a *Lophius*, the plasma Mg of which was lower than that of the pool of blood contained in the reservoir, the first two samples of urine being a wash-out.

Owing to the progressive exhaustion of the Mg reserve in the plasma, there is a regular drop of Mg, down to 0·47 mg % at the fifth hour of perfusion. With a delay of about 80 min, there is a parallel drop in the Mg concentration in urine and in plasma. In 80 min each kidney gives ± 2·5 ml. urine. When a total amount of 15 ml. of urine have been excreted by the two kidneys, the plasma Mg has dropped to 0·47 mg %, showing that excretion continues even when practically all the available Mg has been removed. Some blood was lost during the perfusion, so that it is impossible to compare the available Mg with the excreted amount.

In Expt. 54C (Fig. 2) the initial level of Mg in the plasma was 13 mg %, with a pool of blood of 300 ml., and a red-cell volume of 15·8%. (Loss of blood during perfusion: 10 ml., sampling: 30 ml., total: 40 ml.)

After perfusing two kidneys weighing 12 and 11 g respectively, for 4 h 37 min, the Mg level in the plasma is down to 0·9 mg; there is a parallel drop of Mg in the urine (one kidney only is represented in the diagram) with a delay of 140 min. Of the 33·8 mg of Mg contained in the pool of blood (minus the losses and samples), 29·1 mg had been excreted after that time; thus ± 4·7 mg are left in about 218 ml. of plasma, which would represent 2·1 mg % against 0·9 actually found. This means an error of 2·6 mg in all measurements and determinations involved. 73 min later, when two more ml.

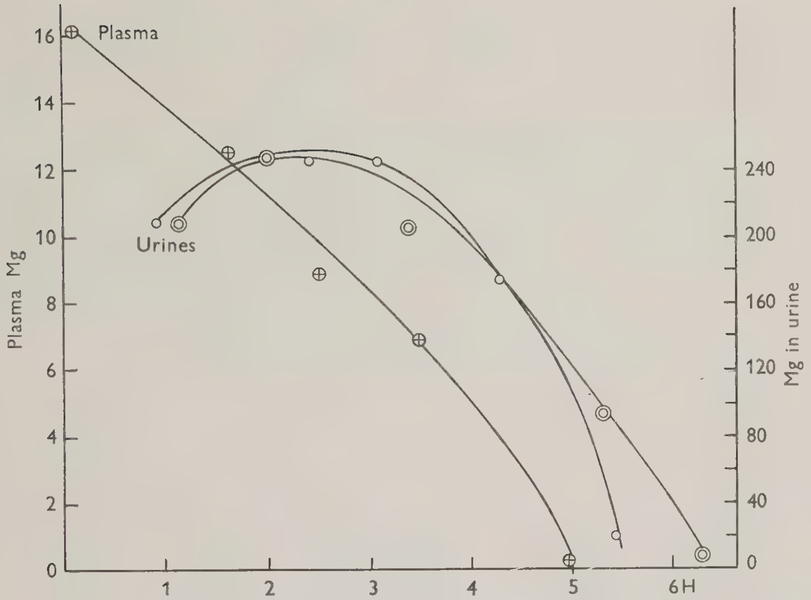

Fig. 1. Two kidneys are perfused until the plasma Mg is exhausted. All figures are given in mg per 100 ml.

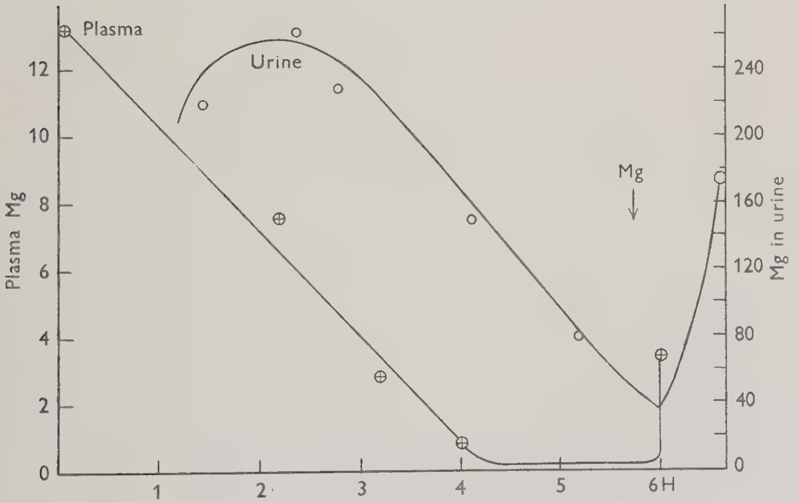

Fig. 2. Two kidneys are perfused until the plasma Mg drops down to 0·9 mg %. After addition of Mg sulfate to the blood, there is a rise of Mg in the urine.

of urine have been excreted, representing ±2 mg of Mg, and the total available Mg being exhausted, Mg is added to raise the plasma level up to 3·3 mg %. In the next sample there is an important rise in urinary concentration of Mg.

Expt. 54L (Fig. 3) starts with a pool of blood of 260 ml., a red-cell volume of 8%, and a Mg content in the plasma of 10·1 mg. Two kidneys of 13 and 11 g are perfused for 4 h. After 3 h of perfusion, 20·6 mg were excreted, while there were ±22·2 mg available (considering the sampling). The plasma content was found to be 0·225 mg %, instead of 1·1 theoretically. However, the kidneys kept on excreting Mg until it was practically exhausted. Mg was added (in the form of sulfate), raising the plasma level up to 2·5 mg with an immediate rise in urine Mg, and a new drop of the plasma level.

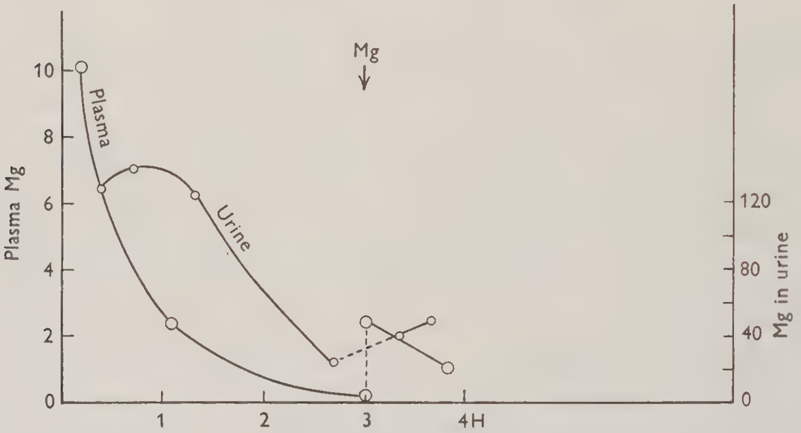

Fig. 3. Two kidneys are perfused until the plasma Mg drops from 10·1 to 0·225 mg %. Addition of Mg sulfate to the plasma produces a rise in urinary Mg.

General Discussion

The normal physiological plasma concentration of Mg has not been established in *Lophius*. In a former paper (Brull & Cuypers, 1954) we gave figures showing an average of 12·1 mg/100 ml. (from 6·32 up to 16·7), in fish bled on arrival in the laboratory, and also one figure of 6·5 mg in a fish bled on board immediately after capture. Other figures found in 1954 aboard the *Sarsia*, after immediate bleeding, are: 2·6, 3·2, 7·2, 3·4, 8·6, giving an average of 5 mg, and we must mention one *Lophius* arriving in the laboratory with 1·98 mg.

It is well known that mammalian plasma contains an average Mg content of about 2 mg/100 ml. It is likely that while the fish are being trawled, unusual quantities of sea water are swallowed and absorbed, thereby increasing the Mg concentration in the plasma on the way to the laboratory. We may however suppose, from the above figures, that the normal concentration is about 2 mg or less.

As long as the plasma figures are higher than, let us say, 5 mg, the percentages in urine are high, up to 200 or even nearly 300 mg/100 ml. This concentration seems to be the maximum attainable for Mg concentration by *Lophius* kidneys. At first sight, when we performed perfusions with high initial plasma figures, and lasting only for 3–4 h, it looked as if urinary percentages of Mg were independent of the plasma concentration; this is true down to a certain level. But it is clear, from the three experiments reported, that when the plasma Mg drops down to 4, 3 or 2 mg, there is a parallel drop in urinary percentage.

But the most striking fact of all is that, while we believe we may consider Mg as a normal constituent of the plasma, the perfused *Lophius* kidneys keep on excreting Mg until its total, or practically total exhaustion, as if there were no threshold.

In our opinion, this finding is capable of two explanations. (1) The isolated perfused kidney of *Lophius piscatorius* lacks some (hormonal?) control to maintain its Mg threshold. (2) *Lophius* is a very greedy fish in the stomach of which enormous amounts of sea animal food can be found; sea water may be unavoidably ingested with the food, thus producing a continuous inflow of Mg, the latter to be neutralized by a very active power of excretion the kidney possesses. No mechanism for the preservation of Mg is required. On the contrary, the problem of its constant removal is by far more urgent. The plasma level may be more variable than in terrestrial or freshwater animals, in which Mg may eventually be lacking.

Under normal conditions of life, a minimum amount of sea water is swallowed, and the excess Mg is easily excreted by the kidneys. After trawling, there is a surfeit of Mg in the blood, representing up to 16 or 17 mg %.

As far as we know, in mammals, there is a very accurate homeostatic mechanism for minerals. One of us demonstrated in 1927 a threshold for phosphates (Brull, 1927), which is under control of the hypophysis (Brull, 1928), and of the parathyroids (Brull, 1936). This author also produced evidence that usually protein-bound and ionized Ca is not excreted by the kidney, but that a small quantity of Ca combined in a complex form with organic acids, is the origin of the urinary leakage of Ca (Brull, 1930).

It is the first time we come across conditions under which a normal inorganic constituent of the plasma is excreted by the kidney until exhaustion. Further research will have to show whether this phenomenon, observed in the perfused kidney, may be confirmed with the whole animal.

Moreover, exogenous Mg, added to the plasma, seems to be excreted in the same way as absorbed Mg.

SUMMARY

Kidneys of *Lophius piscatorius*, perfused with heparinized *Lophius* blood, concentrate magnesium up to one hundred times. This degree of concentration is independent of the plasma level as long as the blood contains high amounts of magnesium, as occurs in the hours following capture of the fish. Below 5 mg % or less, there is a parallel drop in urinary concentration of magnesium and in plasma level. When kidneys are perfused with a limited pool of blood, they excrete magnesium until total exhaustion of this substance. Under such conditions there is no threshold for magnesium in *Lophius*. Exogenous magnesium added to the plasma is excreted in the same manner as endogenous (absorbed) magnesium.

We are much indebted to the Marine Biological Laboratory, Plymouth, for all facilities provided year after year for our research.

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THE GROWTH RATE OF THE LONG ROUGH DAB *HIPPOGLOSSOIDES PLATESSOIDES* (FABR.)—A CORRECTION

By T. B. Bagenal

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(Text-fig. 1)

In a recent paper (Bagenal, 1955) on the growth rate of the Long Rough Dab *Hippoglossoides platessoides* (Fabr.) in the Clyde Sea area, a serious mistake was made.

The ages of 1561 Long Rough Dabs, which were collected from October 1953 to September 1954, were determined from an examination of the otoliths, and from the age analysis each fish was allocated to a population *f* to *a*, depending on when it was supposed to have spawned. This procedure was designed to obviate the difficulty during the winter and spring when fish of the same year-class would be grouped differently according to the condition of the otolith edge. The mean lengths of the fish of populations *f–a* were taken as the mean lengths of Long Rough Dabs after 1–6 years. This, however, is wrong, and they should be taken as the mean lengths after 2–7 years. I wish to apologize for this inexcusable mistake, and to give the correct figures at the earliest opportunity. They will be found in Table I.

Turning next to the mathematical treatment of the data, and applying the correct figures to the same formulae as were used before, it is apparent that the regression equation

$$l_{n+1}=ml_n+C, \qquad \text{(i)}$$

where l_n is the mean length at age n, will be exactly the same, as will the asymptotic value, l_∞, to which the growth approaches. This is because the regression is based on the l_{n+1} to l_n ratio, and the actual values of n are immaterial. The figures obtained for m and l_∞ $(=C/1-m)$ may again be incorporated in the exponential equation modified from Bertalanffy (1938, 1949):

$$l_t=l_\infty\,(1-e^{-kt}), \qquad \text{(ii)}$$

where $e^{-k}=m$ of equation (i). However, with the correct figures there is a large discrepancy between the values of C and what would be reasonable figures for l_1; for equation (i) and (ii) to be comparable these should be the same:

for the females $C=11{\cdot}262$ cm, the calculated $l_1=2{\cdot}130$ cm,
for the males $C=7{\cdot}368$ cm, the calculated $l_1=5{\cdot}335$ cm.

It is clear, therefore that the growth is only described by the equations given in the previous paper over the upper range of the curve, and in order to describe the growth over the whole life span a sigmoid curve which above the point of inflexion approximates to the exponential type given in equation (ii) is required.

TABLE I. THE MEAN LENGTHS OF LONG ROUGH DABS, FROM POPULATIONS *f–a*

Population	Age	Mean lengths (cm.)	
		Males	Females
—	1	—	—
f	2	10·5	12·6
e	3	13·5	18·3
d	4	15·3	22·1
c	5	16·3	23·4
b	6	—	25·3
a	7	—	25·3

Of the commoner growth equations the most appropriate would appear to be the Gompertz equation whose properties have been given by Winsor (1932). This equation has the form

$$l_t = l_\infty \ \exp[-be^{-kt}]. \qquad \text{(iii)}$$

Its relation to equation (ii) is seen by considering the graph of l_{n+1} plotted against l_n. With the Gompertz equation one obtains a curve which cuts the 45° line at the origin and at l_∞, and a straight line regression can be got by plotting the logarithms of the lengths. In this case the regression is

$$\log l_{n+1} = m' \log l_n + C'. \qquad \text{(iv)}$$

The value of l_∞ is obtained from the equation

$$\log l_\infty = \frac{C'}{(1-m')}. \qquad \text{(v)}$$

The slope of the curve on the simple $l_{n+1} - l_n$ plane at any fraction $(1/x)$ of l_∞ is equal to $\dfrac{m'}{x^{m'-1}}$. The curve rises rapidly from the origin and at its intersect with the 45° line has the same slope as that for equation (iv). It is therefore apparent that the two curves are indeed similar, particularly at their upper ranges when they approach their asymptotic levels.

Applying equation (iv) to the Long Rough Dab data of Table I gives

$$\text{for the females: } \log l_{n+1} = 0{\cdot}475 \log l_n + 0{\cdot}742,$$

$$\text{for the males: } \log l_{n+1} = 0{\cdot}501 \log l_n + 0{\cdot}619,$$

and equation (v) gives:

$$\text{for the females: } \log l_\infty = \frac{0{\cdot}742}{(1-0{\cdot}475)} = 1{\cdot}4133 \,.\, l_\infty = 25{\cdot}90,$$

$$\text{for the males: } \log l_\infty = \frac{0{\cdot}619}{(1-0{\cdot}501)} = 1{\cdot}2405 \,.\, l_\infty = 17{\cdot}40.$$

These may be compared with 26·437 and 17·754 for the females and males respectively given in the previous paper for equation (ii). The value for b for the curve to pass through the origin is given by

$$b = \frac{\log l_\infty}{\log e}, \tag{vi}$$

$$\text{for the females: } b = \frac{1{\cdot}4133}{0{\cdot}4343} = 3{\cdot}2542,$$

$$\text{for the males: } b = \frac{1{\cdot}2405}{0{\cdot}4343} = 2{\cdot}8563,$$

and e^{-k} equals m' of equation (iv). The Gompertz equations (iii), for the Long Rough Dabs, are therefore

$$\left.\begin{array}{ll} \text{for the females:} & l_t = 25{\cdot}90 \,.\, 2{\cdot}7183^{-3{\cdot}2542 \,.\, 0{\cdot}475^t} \\ \text{for the males:} & l_t = 17{\cdot}40 \,.\, 2{\cdot}7183^{-2{\cdot}8563 \,.\, 0{\cdot}501^t} \end{array}\right\} \tag{vii}$$

The calculated values from these equations are given in Table II, and in Fig. 1.

TABLE II. THE OBSERVED MEAN LENGTHS OF THE LONG ROUGH DABS, AND THE VALUES CALCULATED FROM THE GOMPERTZ EQUATION (vii)

	Females		Males	
t	Observed	Calculated	Observed	Calculated
1	—	5·52	—	4·16
2	12·6	12·43	10·5	8·50
3	18·3	18·27	13·5	12·14
4	22·1	21·95	15·3	14·87
5	23·4	23·94	16·3	15·90
6	25·3	24·96	—	—
7	25·3	25·44	—	—

In the previous paper it was argued that the calculated mean lengths are less liable to bias (as might, for example, be produced by net selection) than are the observed values. This is because the regression equation is based on the ratios of the lengths and not on their absolute values. The same argument applies to the present treatment with the $\log l_{n+1} - \log l_n$ plane and the Gompertz curve. It will be seen that the agreement between the observed and calculated values is remarkably good with the females, but there is an appreciable discrepancy with the males. The reason for this is not readily apparent, and it can only be suggested that different reactions of the fish to the trawl might be responsible, in leading to greater escape by the males. In this connexion one should remember that there is a marked difference between the numbers of males and females caught; even in comparable size-groups the females are much more numerous.

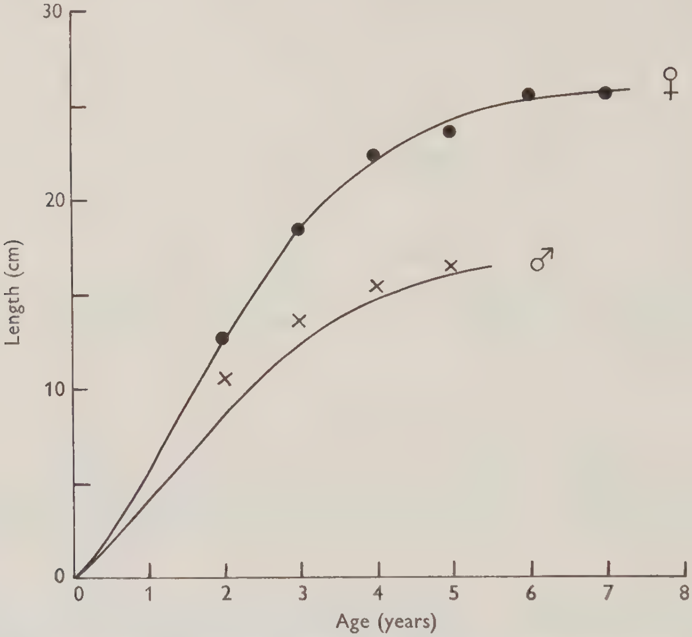

Fig. 1. The growth curves of male and female Long Rough Dabs given by the Gompertz equations (viii), together with the observed mean lengths for the males (×) and females (●). Data from Table II.

SUMMARY

A serious mistake in a previous paper on the growth rate of the Long Rough Dab *Hippoglossoides platessoides* (Fabr.) is corrected. The growth equation originally used is shown to be inapplicable to the correct figures over the whole life span, though for suitable data the method of fitting the curve would still appear to be the most accurate. More suitable for the correct Long Rough Dab data is the Gompertz equation, and this approximates to the previous equation over the upper ranges.

The method previously developed of finding estimates of the coefficients to be used for the growth curve is shown to be equally applicable to the Gompertz

equation. The agreement between the calculated and observed values is very close with the females, but less so with the males. It is suggested that this may in part be connected with the scarcity of the males in the samples, since a greater ability to escape would produce both effects.

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ABSTRACTS OF MEMOIRS

RECORDING WORK DONE AT THE PLYMOUTH LABORATORY

PYTHIUM THALASSIUM N.SP. INFECTING THE EGG-MASS OF THE PEA-CRAB, *PINNOTHERES PISUM*

By **D. Atkins**

Trans. Brit. mycol. Soc., Vol. 38, 1955, pp. 31–46

A marine *Pythium*, *P. thalassium* n.sp., has been found parasitic and saprophytic in the eggs of the pea-crab, *Pinnotheres pisum*, and of other Crustacea. The sporangia are filamentous, the method of asexual reproduction is that characteristic of the genus *Pythium*: proliferation occurs. Some variation in the behaviour of the contents of the sporangium was noted. Asexual bodies, similar to those described in certain other species of *Pythium*, are borne extramatrically at the apex of hyphae. Various stages in the germination of spores were seen. Sexual organs were not present in the material examined. The affinities of *P. thalassium* are discussed. D.A.

THE POST-EMBRYONIC DEVELOPMENT OF BRITISH *PINNOTHERES* (CRUSTACEA)

By **D. Atkins**

Proc. zool. Soc. Lond., Vol. 124, 1955, pp. 687–715

The two species of *Pinnotheres* occurring at Plymouth have been reared in the laboratory from egg to megalopa for the first time.

P. pisum has four zoeal stages and a megalopa. The megalopa stage was reached in 11 weeks from spawning and 6 from hatching. These times are probably considerably longer than under natural conditions.

P. pinnotheres has two zoeal stages and a megalopa. The megalopa stage was reached in about 9 weeks from early egg and three weeks from hatching.

The stages are described and some observations are given on habits and moulting of the zoeae. D.A.

FIRST-STAGE LARVAE HATCHED FROM NEW ZEALAND DECAPOD CRUSTACEA

By **M. V. Lebour**

Ann. Mag. nat. Hist., Ser. 12, Vol. 8, 1955, pp. 43–48

Larvae hatched by Dr E. Batham at the Portobello Marine Laboratory, New Zealand, were sent to the author and are here described. These are *Palaemon affinis*, *Alope spinifrons* and *Callianassa (Trypaea) filholi*. No *Alope* larva has as yet been described. These are very like *Hippolyte* with a few minor differences. The larva of *Callianassa filholi* is here described for the first time. It is peculiar in the armature of the abdomen and in this respect unlike any species known. M.V.L.

OBSERVATIONS ON THE FOOD AND THE GUT PIGMENT OF THE POLYOPISTHOCOTYLEA (TREMATODA: MONOGENEA)

By **J. Llewellyn**

Parasitology, Vol. 44, 1954, pp. 428–37

The Polyopisthocotylea is a suborder (upwards of 250 species) of monogenetic trematodes that are parasitic mainly on the gills of fishes, but the nature of the food of these parasites has not previously been determined.

An investigation by spectroscopic and histochemical methods of six representative trematodes from the gills of marine fishes at Plymouth and of trout from elsewhere, and of the monogenean parasitic in the bladder of the frog, has shown that these parasites feed mainly on the blood of their hosts. The blood is probably haemolysed fairly rapidly and subsequently absorbed by amoeboid ingestion, the globin moiety of the haemoglobin forming the chief nutriment of the parasite and the unaltered haematin being eliminated either by its discharge from epithelial cells into the gut lumen or by the sloughing off of intact epithelial cells.

In a more limited sample of skin- and cloaca-inhabiting Monopisthocotylea there was no evidence of a blood-feeding habit. J.L.

THE STRUCTURE AND FUNCTION OF THE BRITISH PHOLADIDAE (ROCKBORING LAMELLIBRANCHIA)

By **R. D. Purchon**

Proc. zool. Soc. Lond., Vol. 124, 1955, pp. 859–911

Exhaustive study of the Pholadidae, the least specialized family in the Adesmacea, revealed two features of potential systematic importance. The appendix of the stomach is homologous with the caecum of the Teredinidae and with the postero-dorsal caecum of the Tellinacea, thus indicating relationship between the Adesmacea and the Tellinacea. An accessory visceral ganglion is present in the Pholadidae, and such a structure has been reported elsewhere only in *Dreissena*.

When all other ciliary activity appeared normal, cilia in the proximal oral groove were usually inactive, suggesting that feeding is intermittent. It was concluded that the ciliary mechanisms in the stomach served chiefly for trapping and rejection of particles, and that food material could not be passed from the stomach into the digestive diverticula solely by ciliary action. Probably during a period of ciliary inactivity in the proximal oral groove, the stomach contents are prepared for presentation to the digestive diverticula, during which time the stomach contents are not contaminated by entry of fresh food material. Finally the stomach contents are passed to the digestive diverticula by relative volumetric changes in the stomach and digestive diverticula. These volumetric changes are brought about by the adductor and pedal muscles, and by the muscle fibres in the walls of the stomach and digestive diverticula. R.D.P.

Growth Changes in the myelin Sheath of peripheral nerve Fibres in Fishes

By **P. K. Thomas**

Proc. roy. Soc. B, Vol. 143, 1955, pp. 380–391

A quantitative study of the growth of the myelin sheath of fibres of the lateral line nerve in *Salmo trutta* and *Raia clavata* has been made. This nerve is especially favourable for growth studies because of the magnitude of the changes taking place. In a large specimen of *Salmo*, the largest fibres attain a diameter in excess of 30μ and possess internodes 4 mm in length. In *Raia*, internodes of up to 8 mm in length are found.

Contrary to earlier reports, but in agreement with a recent investigation on mammalian nerves, the relative thickness of the myelin sheath is found to decrease progressively during growth as fibre size increases. Comparison between fishes of differing length demonstrates that the thickness of the myelin sheath for a given axon diameter is constant, irrespective of the size of the fish.

Measurement of the internodal length of the largest fibres in the nerve in specimens of differing length confirms the close dependence of the length of the myelin segments on growth. The form of the relationship between internodal length and fibre diameter depends upon their relative growth rates. Growth of internodal distance follows that of the part in which the nerve lies, but this is not true of diameter. The changes in the relationship between internodal length and diameter during growth have been analysed and the applicability of the equation employed to other vertebrate groups considered.

The contribution that quantitative investigations of the changes taking place during the growth of nerve fibres can make towards the understanding of the way in which their structural organization is achieved and maintained is discussed. P.K.T.

THE DISTRIBUTION OF CERTAIN INTERTIDAL ANIMALS AROUND THE IRISH COAST

By **A. J. Southward** and **D. J. Crisp**

Proc. R. Irish Acad., B, Vol. 57, No. 1, 29 pp.

During the period 1950–53, the distribution of some animals found mainly on the upper half of the shore was investigated at ninety-six stations round the Irish Coast.

The barnacle *Chthamalus stellatus* was found on exposed shores of all coasts, except for occasional areas of chalk in Co. Antrim, and was the dominant barnacle of the south and west coasts. It was generally less common on the east coast, but was quite abundant in a limited area of the coasts of S. Co. Down and Co. Louth. *Balanus balanoides*, on the other hand, was practically absent from the extreme South-west, its distribution being almost the inverse of that of *Chthamalus*.

The top-shells *Gibbula umbilicalis* and *Osilinus lineatus* followed the main features in the distribution of *Chthamalus*, but *Osilinus* was absent from most of the north and east coasts except for an area in S. Co. Down and Co. Louth. The sea-urchin *Paracentrotus lividus* was found only on the west coast.

Of several other animals investigated, the parasite *Hemioniscus balani* was more common in the South-west, and *Littorina neritoides*, though common on all exposed shores, was generally less abundant on the east coast.

The distributions of the southern species, notably *Chthamalus*, *Osilinus*, *Gibbula umbilicalis* and *Paracentrotus* have a common basis, apparently the effect of temperature rather than any other known or unknown factor in the environment. In some of these animals the effect of low summer temperatures on the breeding activities must restrict the distribution in certain areas. The anomalous abundance of *Chthamalus*, *Osilinus* and *Gibbula umbilicalis* on a limited part of the east coast may be due to a combination of local warming during the summer, with suitable rock topography and tidal conditions. Similar examples of the possible beneficial examples of local warming during the summer are given in the case of Lough Ine (for *Paracentrotus*), and the Scottish Lochs Sween and W. Tarbert (for *Chthamalus*). A.J.S.

BOOK REVIEWS

ASPECTS OF DEEP-SEA BIOLOGY

By **N. B. Marshall**

Published by Hutchinson's Scientific and Technical Publications, London. Pp. 380. Illustrated by Olga Marshall; with 5 coloured plates, and many black and white illustrations. Price 35*s*. 1954.

In seeking to understand the activities of deep-sea organisms, one is impressed by the scantiness of our present knowledge and the host of unsolved problems which life in the depths presents. In this book Mr Marshall has given a clear and factual account of environmental conditions in the deep sea, and the ways in which plants and animals live in deep waters. It is so written that it can be understood by the non-scientific reader and yet prove of interest and value to professional biologists. After describing background conditions in the sea the author devotes special attention to certain subjects, among which may be mentioned: food chains, counter-acting gravity, sensory perception, sound production, luminescence and reproduction. A great many scattered observations are assembled and are employed to interpret how deep-sea organisms adjust themselves to life at different levels in the open oceans. Most of this information is necessarily indirect for obvious reasons. Not the least interesting feature of the book is the way in which information is assembled from many different branches of biology, when capable of throwing light on the problems under discussion. Many of the problems raised cannot yet be answered, but it is useful to have them clearly stated.

There is a short list of the references at the end of each chapter and the book is amply illustrated by line blocks, half-tones, and coloured plates.

J. A. C. N.

THE INDIAN JOURNAL OF FISHERIES

Vol. 1, Nos. 1 and 2, May 1954. Price 7 rupees 8 annas net.

It is unusual, in these days, to find oneself welcoming the appearance of yet another scientific journal or other scientific periodical. But to the new *Indian Journal of Fisheries* a welcome must be accorded. It is published by the Indian Ministry of Food and Agriculture to provide a medium for the publication of original contributions and critical reviews in the field of Indian fisheries science. Each volume is planned to consist of about 400 pages to be issued in two numbers. The proposed intervals between publication are not stated.

Volume 1, nos. 1 and 2, has already appeared and contains many papers covering a wide range of subjects. The editorial board appears to have set a high standard which it will no doubt maintain. Fisheries research and development in India are now being pursued with such vigour and success that a special publication solely concerned with these subjects has become essential. We therefore extend a hearty welcome to the new *Journal* and wish it every success.

G. A. S.

BIOLOGY OF A MARINE COPEPOD

By S. M. Marshall, D.Sc., F.R.S.E. and A. P. Orr, M.A., D.Sc., F.R.S.E.

Published by Oliver & Boyd, Ltd., Edinburgh. Pp. 1–188, with 63 figs. Price 21*s*. 1955.

This is a very valuable book. *Calanus* is one of the most ubiquitous and abundant animals in the plankton. It is a main source of food for whales and fish, and plays a large part itself in the utilization of the phyto-plankton. It is also a representative of the largest group of Crustacea in the plankton. It is natural, therefore, that more research has been done on the biology of this copepod than on that of any other. Dr S. M. Marshall and Dr A. P. Orr have brought together all existing knowledge on every aspect of the biology of this copepod. They cover its systematics and anatomy, its reproduction and life cycle, and the general physiology of its growth, feeding and respiration, together with information on its behaviour and its parasites. In all of these aspects of the biology of *Calanus* both Dr Marshall and Dr Orr have themselves contributed much to our knowledge by their own researches, which have covered a period of more than thirty years. This book should be of interest not only to those engaged in plankton research, but to all biologists, since it covers problems of fundamental importance concerning life in the sea. The authors are to be congratulated on making available to their fellow workers in so interesting a manner and over so wide a field our knowledge to date on the biology of *Calanus*.

The book is well illustrated both with line and half-tone figures, and is in every way a most pleasing production.

F. S. RUSSELL

MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

Report of the Council for 1954–55

The Council have to record with great regret the deaths of Dr E. S. Russell, O.B.E., a former member of Council and a Vice-President from 1948, of Prof. F. E. Fritsch, F.R.S., also a former member of Council, and of Miss M. J. Delap, an Associate Member since 1937.

During the year Major E. G. Christie-Miller, our Honorary Treasurer, has been elected a Vice-President.

The Council and Officers

Four meetings of the Council have been held during the year, three in the rooms of the Royal Society and one at Plymouth. At these the average attendance was eighteen. The Association is indebted to the Council of the Royal Society for the use of its rooms.

The Plymouth Laboratory

The extension to the library is approaching completion. This new building, which has three floors, will give nearly as much floor space as the existing library. Besides providing extra shelf accommodation the extension will give much needed space for reading. The new specimen store has now been finished and is in use.

The Council are pleased to report that H.M. Treasury have sanctioned a Capital Grant from the Development Fund to meet the cost of constructing new sea-water reservoirs and rebuilding and extending the existing outside storage tanks.

The Aquarium

The Aquarium has continued to attract large numbers of visitors, many of whom have remarked favourably on the improvements made last year.

Some additional picture labels have been painted by Miss Jenefer Peter so that most, though not yet all, of the animals shown can be identified by the visitor.

During October it was possible to show a special exhibit of living Portuguese Men-of-War (*Physalia physalis*), at a time when they were being stranded on the shores of south-west England.

Research Ships

During the year R.V. *Sarsia* has been in continuous service and is giving great satisfaction. The Laboratory is deriving great benefit from her greater range of activities which enable her, among other things, to bring back living organisms from much farther afield and from greater depths.

Sula has undergone Lloyds ordinary special hull survey and the hull has been found to be in excellent condition. Unfortunately, routine inspection of the machinery revealed unexpectedly heavy wear of the main bearings of the engine and in the gear box. Lloyds therefore ordered a complete overhaul of the machinery. This has been carried out at considerable expense.

Gammarus has worked normally throughout the year.

The Staff

Dr W. R. G. Atkins, C.B.E., F.R.S., retired from the Staff of the Plymouth laboratory on 31 March 1955. The Council wish to record their deep appreciation of Dr Atkins's services to the Association as Head of the Department of General Physiology for 34 years, and of his own distinguished research which has gained for the Plymouth laboratory an international reputation. They wish him many years of happy retirement.

Miss D. Ballantine joined the staff of the Plymouth Laboratory in the grade of Scientific Officer, on 1 October 1954.

Dr G. A. Steven attended the meeting of the International Council for the Exploration of the Sea in Paris in October 1954.

Dr D. P. Wilson spent the month of October 1954 at Naples by invitation of Dr R. Dohrn to discuss improvements in the sea-water circulation at the Stazione Zoologica.

Dr L. H. N. Cooper attended the opening of the new Laboratory of Oceanography of the U.S. Office of Naval Research at Woods Hole in June 1954, and read a paper at a subsequent convocation. While in America Dr Cooper visited the Fish and Wild Life Service Laboratories at Galveston in Texas, and at Stanford in California, the marine laboratories at Miami, the Scripps Institution of Oceanography, and Yale University.

In September 1954 Dr L. H. N. Cooper attended the meeting of the International Union of Geodesy and Geophysics in Rome, after which he spent a few days at Monaco and Villefranche-sur-Mer.

Dr Mary Parke attended the VIIIth International Botanical Congress in Paris in July 1954, where for the first time meetings of a Phycological section were held. After the Paris meeting she spent a few days at Roscoff.

Mr P. G. Corbin accompanied the cruise of the R.R.S. *Discovery II* in September 1954, by kind invitation of the Director of the National Institute of Oceanography, to test his large pelagic net.

Dr J. A. C. Nicol returned to Plymouth in September 1954 after spending twelve months in the United States, while holding a Guggenheim Fellowship. The greater part of this time was spent at the Scripps Institution of Oceanography, but Dr Nicol also visited Santa Barbara and Friday Harbour.

Mr F. S. Russell, F.R.S., was appointed a Commander of the Order of the British Empire in the New Year's Honours for 1955.

International Paints Research Fellowship

The Directors of International Paints Ltd. have, by arrangement with the Council of the Association, endowed a research fellowship for investigation of the effects of toxic substances on marine organisms. This is to be known as the International Paints Research Fellowship and is tenable for three years at the Plymouth laboratory.

Dr E. D. S. Corner, of the Department of Biochemistry, St Thomas's Hospital Medical School, has been appointed to the Fellowship, and he started work at Plymouth on 1 September 1954.

Occupation of Tables

The following one hundred and fifty-seven workers have occupied tables at the Plymouth Laboratory during the year:

E. Adams, Plymouth (Library).
Miss J. M. Allen, London (General).
Señora A. A. Alvariño, Madrid, British Council Scholar (Plankton).
D. C. Arnold, Oxford (Biology of Carapidae).
Miss D. E. Ashhurst, Oxford (Blood groups in fishes).
Dr Daphne Atkins, London (Ciliary feeding mechanisms of brachiopods and *Phoronis*).

Miss D. Ballantine, Development Commission (Taxonomy and culture of marine flagellates).
Miss S. Baur, Basel (Scientific drawings).
D. S. Bendall, Cambridge (Chromatography of nucleotides).
Dr Anna M. Bidder, Cambridge (Library).
Dr H. M. Bishai, Cullercoats (Biology of fish larvae).
Dr H. Blaschko, Oxford (Amine metabolism in invertebrates).
B. Boczarow, Plymouth (Library).
A. D. Boney, Plymouth (Shore ecology).
Prof. A. A. Boyden, New Jersey (Comparative serology).
L. R. Brightwell, Peacehaven, Sussex (Biology of pagurids).
Dr L. G. Brock, Cambridge (Mode of excitation of electric organ in *Raia*).
Dr Eleanor M. Brown, London (Digestive system of mysids and *Nebalia*; *Syndinium*).
Prof. & Mme L. H. Brull, Liége (Physiology of kidney in *Lophius*).
Dr P. Brunet, Oxford (Byssus of *Mytilus*).
Dr M. Buljan, Split (Chemical hydrography).

Dr P. C. Caldwell, London (Giant nerve fibres of *Loligo*).
Dr J. N. Carruthers, National Institute of Oceanography (Testing oceanographic equipment).
Dr J. D. Carthy, London (Biology of larval *Geranomya*).
P. Childs, Plymouth (Comparative anatomy of biliary tract).
B. I. Clarke, Leeds (Marine flagellates).
R. D. Cohen, Plymouth (Library).
Dr H. A. Cole, Burnham-on-Crouch (Oyster fisheries).
D. J. Collins, Plymouth (Library).
R. J. Conover, Southampton (General).
Dr E. D. S. Corner, International Paints Research Fellow (Effects of toxic substances on marine organisms).
C. A. Cosway, Torquay (Library).
Miss M. Courtney, London (Cirratulids).
Dr Y. Cuypers, Liége (Physiology of kidney in *Lophius*).

Dr R. Phillips Dales, London (Feeding and morphology of the gut of terebellid polychaetes).
R. D. Deshpande, Southampton (Biology of *Acartia*; ecology of Trochidae).
Mr and Mrs P. S. B. Digby, London (Feeding of copepods).
Miss E. J. Dimelow, Reading (Functional morphology of *Antedon*).
D. A. Dorsett, London (Digestive cycles in *Gibbula*).
Mme M. Dupont-Raabe, Paris (Colour change in insects and crustaceans).

C. Edwards, Belfast (Ecology of *Marinogammarus*).
J. H. Elgood, Rustington (Library).
S. K. Eltringham, Southampton (Biology of *Limnoria*).

R. W. Fearnhead, Cambridge (Development of fish teeth).
Dr Maria Felinska, Buckden, Huntingdon (Ciliates).
Dr L. R. Fisher, Reading (Vitamin A and carotenoids).
Dr E. W. Flückiger, Basel (Giant nerve fibres of *Loligo*).
A. R. Fontaine, Oxford (Feeding of ophiuroids).
B. A. Foster, Aberdeen (Culture of marine flagellates).
Cdr R. H. C. Frampton, R.N. (Rtd.), Plymouth (Library).
Dr. C. B. Frankenhaeuser, Stockholm (Giant nerve fibres in *Loligo*).
Dr Vera Fretter, London (Uptake of ^{24}Na by *Nereis*).

J. B. Gilpin-Brown, Bristol (Nervous system of *Nereis*).
Dr Sylvia J. Gilpin-Brown, Bristol (Behaviour of littoral gastropods).
Dr D. R. Glasson, Plymouth (Library).
R. U. Gooding, Cambridge (Underwater ecology).
Mr & Mrs T. F. Goreau, Jamaica (Phosphates in corals).
Prof. A. Graham, Reading (Library).
A. V. Grimstone, Cambridge (Gastropods).

Miss J. E. Hannaford, Plymouth (Library).
R. E. Harris, Manchester (*Callithamnion*).
B. T. Hepper, Burnham-on-Crouch (Oyster fisheries).
Miss J. M. Himms, Oxford (Amine metabolism in invertebrates).
Miss A. Hinds, Cambridge (Chromatography of nucleotides).
Prof. A. L. Hodgkin, F.R.S., Cambridge (Giant nerve fibres in *Loligo*).

D. B. Hope, Oxford (Amine metabolism in invertebrates).
Dr G. A. Horridge, Cambridge (Ephyra larvae).
G. Hoyle, Glasgow (Neuromuscular physiology of ascidians).
Dr A. F. W. Hughes, Cambridge (Cytology of fish nervous systems).
Surg.-Lt. C. O. Hughes, R.N., Alverstoke (Collection of fish juices).
Mrs S. Hughes, Cambridge (Development of fish teeth).

L. A. J. Jackman, Paignton (Library).
F. J. Jeffery, Plymouth (Library).
Dr C. H. Jellard, Plymouth (Bacteriological tests).
Dr Penelope M. Jenkin, Bristol (Feeding of flamingoes; Library).

Miss J. M. Kain, London (Culture of dinoflagellates).
Dr G. Y. Kennedy, Sheffield (Porphyrins in marine animals).
Dr R. D. Keynes, Cambridge (Giant nerve fibres in *Loligo*).
M. C. Kingwell, South Brent (Library).
P. Kirtisinghe, Ceylon (Parasitic copepods).
Sir Francis Knowles, Bart., Marlborough (Neurosecretion in crustaceans).
Dr S. K. Kon, Reading (Vitamin A and carotenoids).
M. E. Korn, London (Holothurians).
Dr P. L. Kramp, Copenhagen (Hydroids and medusae).
Prof. E. Krogh, Aarhus (Nucleic acid in *Aplysia* nerve cells).

Dr Marie V. Lebour, Plymouth (Decapod crustaceans).
H. Leivestad, Bergen (Endocrinology).
D. Leston, London (*Aëpophilus*).
Dr J. Llewellyn, Birmingham (Trematode ectoparasites of fishes).
A. P. M. Lockwood, Cambridge (Green glands of crustaceans).
P. A. Longton, Plymouth (Library).
Prof. O. E. Lowenstein, F.R.S., Birmingham (Sense physiology of *Raia* and *Motella*).
Dr A. G. Lowndes, Plymouth (Mineral contents of filtered sea water).

Mrs F. J. H. Mackereth, Windermere (Amphipods).
Prof. Irene Manton, Leeds (Marine flagellates).
P. T. Marshall, Cambridge (Library).
Dr Sheina M. Marshall, Millport (Feeding experiments on *Calanus*).
Mlle M. M. Martin, Paris (Marine animal painting).
R. B. Mayne, Plymouth (Library).
J. S. McDonald, Edinburgh (General).
Prof. A. E. W. Miles, London (Development of fish teeth).
P. L. Miller, Cambridge (Gastropods).
Prof. N. Millott, Jamaica (Echinoderm pigments).
Dr J. E. Morton, London (Daily rhythms in *Lasaea*).
Dr R. W. Murray, Birmingham (Sense physiology in elasmobranchs).

Dr G. R. E. Nayler, Cambridge (*Maia* blood).
Dr Margaret Naylor, London (Life histories of Phaeophyceae).
Dr J. Needham, F.R.S., Cambridge (Chromatography of nucleotides).
Miss M. E. Needler, London (Neuromuscular physiology of sea anemones).
B. S. Newell, Zanzibar (Library).
Dr Wheeler J. North, Cambridge and La Jolla (Light sensitivity of *Metridium*).

Dr A. P. Orr, Millport (Feeding experiments on *Calanus*).

Dr C. F. A. Pantin, F.R.S. Cambridge (Light sensitivity of sea anemones).
A. H. Papworth, Northampton (Plankton).
F. G. E. Pautard, Leeds (Locomotive organization in Protozoa).
Prof. B. Peyer, Zürich (General).
D. G. Pollard, Plymouth (Library).
Mme S. Pontus, Liége (Physiology of kidney in *Lophius*).
Dr H. H. Poole, Dublin (Photoelectric measurements of submarine illumination).
Miss E. C. Pope, Sydney (Cirripedes).
L. B. Pradhan, Madras (Mackerel fisheries).

Dr W. J. Rees, London (E. T. Browne Collection of Medusae).
Dr K. Reich, Jerusalem (General).
Miss B. Rickard, Plymouth (Library).
Miss J. E. Rigby, London (Library).
Dr F. H. Rigler, Atomic Energy of Canada, Ltd. (Ion exchange in plant cells).
Miss E. A. Robson, Cambridge (Motor elements in *Metridium*).
Dr D. M. Ross, London (Behaviour in sea anemones).
M. J. S. Rudwick, Cambridge (Brachiopods).

A. Sharples, Plymouth (Library).
Dr E. M. Shelswell, Mitcham, Surrey (Comparative serology).
Dr R. I. Smith, Berkeley, California (Chloride regulation in *Nereis diversicolor*).
R. J. Snell, Ruislip (Pelagic fisheries).
R. E. Soeriaatmadja, Indonesia (Chemical hydrography).
Dr A. J. Southward, D.S.I.R. (Shore ecology).
Mrs A. J. Southward, Plymouth (Polychaetes).
Miss F. A. Stanbury, Plymouth (*Cladophora*).
Miss J. E. Steinberg, California (Opisthobranchs and caprellids).
Dr F. C. Stott, Ewell (*Holothuria*).
G. Sundnes, Bergen (Endocrinology).
J. C. Swallow, National Institute of Oceanography (Geophysical methods).

Dr Olive S. Tattersall, Hayling Island, Hants. (Biology of mysids).
I. M. Thomas, Adelaide (Iodine uptake in lower chordates).
A. K. Totton, London (Morphology of *Physalia*).

E. Ursin, Denmark (Bottom sampling methods).

H. L. Van de Vyver, Louvain (Muscle physiology).
J. I. Visfeldt, Aarhus (Nucleic acid in *Aplysia* nerve cells).

Prof. G. P. Wells, F.R.S., London (*Arenicola*).
Miss J. A. Westfall, Naples (Opisthobranchs).
Dr B. Whittington, London (*Aëpophilus*).
G. Wikkenhauser, London (Testing oceanographic equipment).
Mlle L. Wilsens, Liége (Physiology of kidney in *Lophius*).
A. J. Woods, London (Testing oceanographic equipment).
H. V. Wyatt, Plymouth (*Calyptraea chinensis*).

Among the many scientists who have visited Plymouth during the year to see the general work of the laboratory and to discuss problems with members of the scientific staff, the following have come from overseas: Lt.-Cdr. B. M. Bary, R.N.Z.N., W. Macnae, S. Africa; Dr Ellinor H. Behre, Louisiana; Dr E. S. Deevey, Canada; Miss Mary Samuel, S. India; Prof. W. S. Hoar, Canada; Dr G. J. Hunter, Canada; Dr J. R. Nursall, Alberta; Dr H. Pektas, Turkey; G. A. van Erkel, Utrecht; Prof. B. T. Scheer, California; Prof. A. D. Hasler, Wisconsin; Prof. Isao Motomura, Japan; Dr R. S. Dietz, U.S.A.; C. R. Elsey, Vancouver; B. V. Hamon, Sydney; Dr W. Templeman, Newfoundland; Prof. J. W. Durham, California; Dr M. Buljan, Split; Miss M. I. Garcia, Spain; Mr A. Ben-Turia, Haifa.

The Easter Vacation Courses were conducted by Mr G. M. Spooner and Mr P. G. Corbin, and were attended by forty-two students from the following Universities and University Colleges: Oxford, Cambridge, Aberdeen, London, Liverpool, Sheffield, Reading, Southampton, Aberystwyth, Cardiff, Bangor, Exeter and the Chelsea Polytechnic.

Also during the Easter Vacation Mr R. J. Jones brought a party of eighteen boys from Whitgift School, and Mr K. W. Wilkes brought three boys from Harrow School. Mr J. F. Eggleston brought eleven pupils from Hinckley Grammar School.

A Joint Meeting of the Challenger Society and Representatives from the Marine Laboratories (Development Commissioners Scheme) was held at the Plymouth Laboratory on 1 and 2 July 1954. Members of the staff provided the papers and demonstrations for the meeting, which was attended by about ninety persons.

Scientific work of the Plymouth Laboratory Staff

Sea Water and Plankton

In collaboration with Dr H. H. Poole, who again visited the laboratory, Dr W. R. G. Atkins has worked on a method for measuring the extinction coefficient of light in the sea using two submerged photometers and thus avoiding certain errors inherent in a comparison with a cell in air. The apparatus has now been used in the sea. A method has also been developed for measuring the light at a point on the sea, as opposed to the vertical component of daylight. In this, readings are made with the six cells of the cube photometer and compared with those got with a globe photometer in the ship. The cube photometer has also afforded further information on the angular distribution of submarine light. The use of the globe does away with the use of gimbals, as the incidence of sunlight is always normal. Daylight factors in the laboratory have also been determined, using a pair of globe photometers.

Though selenium rectifier cells have an advantage for use under water in their high sensitivity and colour sensitivity none of them is really stable on account of the inherent properties of selenium; as a result the form of the relation between current and light intensity alters and re-standardizations are necessary. With a view to reverting to vacuum emission cells Mr F. J. Warren and Dr Atkins used an amplifier to extend the depth range of this type of cell and were able to measure blue and green light at 70 m some years ago. After a discussion with Dr Poole it now appears possible to use the amplifier for work near the surface also, as a comparatively 'fool proof' method, and this will be tried.

Further measurements have been made, both in the river and at sea, using the 'balance by depth method' to determine the extinction coefficient. The magnitude of the drift error has also been determined under various conditions with the pressure-operated Admiralty depth-recorder. As a control it has also been tried in windless weather and slack water. On account of the rapidity of working the balance by depth method is but little affected by the drift error, nor does alteration in daylight affect it.

Dr Atkins and Mr Warren have tested the stability of a vacuum emission cell of the CMV6 type exposed on the roof for over three years. No change can be detected, nor has the sodium cell of the Burt type changed during this period. It has been on the roof since 1929 save for some of the war years, during which the fire altered its sensitivity. Selenium rectifier cells were also exposed in specially designed water-cooled cases, the current being limited to about that given by a vacuum cell. All infra-red and some red was removed from the daylight by a filter placed outside the water-cooled vessel. The three different types of cell all deteriorated, though one remained constant for over a year. None could be recommended for use in the tropics, as could the CMV6 it seems—for in its manufacture it is baked at a high temperature, well above the melting-point of sodium.

In response to a request from Prof. Hugh C. Trumble, who had visited Africa on behalf of the International Bank for Reconstruction and Development, Dr Atkins has examined samples of West African river and lake waters. Dr Atkins has also modified the diphenylbenzidine method for the determination of nitrate in waters to reduce the effect of any organic matter present. The reagent is easily prepared, stable, sensitive and the colour produced is stable to light.

Using the 'Code des Couleurs' of Klincksieck and Valette and also Ridgway's plates, Miss P. G. Jenkins and Dr Atkins have been able to provide another method for the identification of water masses by comparison of the colour of mineral matter retained by collodion filters after extraction of the phytoplankton pigments. Examination of the residue on filter disks given by water taken at intervals from the surface to bottom, indicates that the mineral matter originates from the surface, not from the bottom, save probably in the autumn equalization of temperature. It appears to come out in fresh water and to be carried long distances in suspension; thus the colour shown at E1 on certain dates was probably due to water from the River Teign with a mineral content derived from the Permian sandstone near Dawlish. They are indebted to Dr A. G. Lowndes for identifying as microcline, a potash felspar, the material found most abundantly from the 5 m water at E 1 on 29 March 1954. Dr Lowndes is now engaged on a systematic examination of such disk deposits and of material from the bottom. The application of petrological methods to marine suspensions and deposits may be expected to give valuable information.

Dr H. H. Poole and Dr Atkins have continued the study of the scattering of light due to suspended matter, and of the effect of the colour of the light. The flask has now been blackened in an attempt to minimize reflexion errors, which are important in their effect upon the residual back scatter. Alterations in design have also been planned for the same purpose. A number of depth series from station E 1 have been examined to follow the seasonal changes. The importance of the small back-scatter arises from its value as an indication of the opacity of the suspension, for opaque obviously reflect more than do translucent particles. As an offshoot from this work on scattering Mr Warren has been able to supply Dr J. A. C. Nicol with helpful information about the vagaries of the photo-multiplier cell and the conditions required for obtaining reliable results.

Miss Jenkins continued her work on the phytoplankton at station E 1. The chlorophyll was determined spectro-photometrically as before. The winter and June minima were quantitatively in close agreement, though the times of occurrence varied. Rough weather prevented observations at the time when the spring outburst was at its maximum in 1954. The years differed remarkably in some respects. Thus *Thalassiosira decipiens*, absent in 1952, was abundant in late months of 1953, whereas *T. gravida*, common in spring and autumn 1952, with some in the spring of 1953, was altogether absent in the autumn of 1953, but very abundant in the spring of 1954. Also *Chaetoceros debilis* was common later in 1952 and 1953, absent in the spring of 1953, and very common in the spring of 1954.

Dr H. W. Harvey has investigated the growth in culture of several species of marine diatoms and flagellates. The addition of vitamin B_{12} (cobalamin) was found necessary to produce considerable population densities of the inshore diatom *Skeletonema costatum* and of two species which are at times abundant at the weather-ship stations in the Atlantic—*Nitzschia seriata* and *Chaetoceras decipiens*. The cells used for insemination had been maintained in cultures containing soil extract, which itself contains this vitamin, but it appears that the cells had not lost their capability of synthesizing the vitamin during the period of culture; a water collected in April, which had a *Skeletonema*-dominant flora, after enrichment and illumination produced few cells without the addition of B_{12}, the addition of which led to dense populations.

Unlike these species of widely distributed planktonic diatoms, *Phaeodactylum* (*Nitzschia closterium* forma *minutissima*), which is very readily cultured and has been used for most of the classical experiments on the physiology of marine phytoplankton, produces very dense population densities without the addition of B_{12}. Also unlike planktonic diatoms, it appears to require very little, perhaps no, silicate, although it rapidly absorbs silicate if this is provided. Another difference is its ability to utilize carbon dioxide in the medium until almost all the bicarbonate is converted to carbonate and the pH has risen to 10·0.

The supply of silicate to *Skeletonema costatum* has been examined. This lightly silicified species, collected off-shore in the spring, contained some 50 times more silica than phosphorus. When grown in enriched sea water with added soil extract, and containing some 2 mg SiO_2 per litre, this diatom rapidly uses the silicate and then becomes silica deficient, further proliferation being then dependent upon the rate of solution of silicate from the glass vessel.

During various attempts to load sea water with sufficient silicate for heavy growths of diatoms without their becoming silica deficient, it was observed that in many sea waters, but not all, *Skeletonema* grew in bent or tangled chains. This could be prevented by the addition of divalent sulphur, as sulphide or thiourea.

This was in line with observations made both here and in Japan which suggested that the deposition of amorphous silica to form the frustules of diatoms is in some way linked with their sulphur metabolism. This conclusion has now been independently arrived at and verified by Mrs Lewin of Halifax, Nova Scotia, using a freshwater pennate diatom.

Experiments on the growth of natural mixed phytoplankton communities in samples of raw water which has been enriched have shown several instances where diatom growth has soon ceased, unless divalent sulphur was also added.

Although these various experiments have shown that natural sea waters may often contain insufficient B_{12} or insufficient 'sulphur factor' for the growth of dense populations of certain diatom species, they provide no evidence of insufficiency affecting the growth rate of the low population densities which occur naturally in the sea.

The co-operation of Mr F. A. J. Armstrong and Mr C. B. Cowey in these experiments is gratefully acknowledged.

Dr Harvey has also made experiments which bear out the conclusion that zooplankton, when feeding heavily on diatoms during the spring outburst and voiding many more broken diatoms than they can digest, may set free material quantities of inorganic phosphate into the euphotic zone, due to dephosphorylation of organic phosphorus compounds by the enzymes set free in the plant tissue when the cells are broken during their passage through the animals.

During his visits to the United States, Italy, France and Monaco, Dr L. H. N. Cooper was able to discuss the similarities between his problems in the Eastern North Atlantic related to biological productivity in Northern

European waters, and those of the Western North Atlantic, and Eastern North Pacific Oceans, the Gulf of Mexico and the Mediterranean. He read a paper at Woods Hole on his investigations on deep-water movements in the North Atlantic as a link between climatic changes around Iceland and the biological productivity of the English Channel and Celtic Sea. This paper will be published in the *Journal of Marine Research*. He is also collaborating with Dr E. G. Goldberg of the Scripps Institution in the preparation of a detailed account of the occurrence of chemical elements in sea water.

Dr Cooper has also been studying the implications on marine research of the draft articles for international legislation on the regime of the high seas prepared by the International Law Commission. A leading article by him appeared in *Nature*, and the subject has since been considered by a number of interested national and international scientific bodies.

Mr F. A. J. Armstrong has continued the regular cruises to station E 1, and analyses for phosphate, total phosphorus and silicon. During spring and summer 1954 extra depths have been sampled. Surface temperatures in this period have been lower than in the past four years, and the silicate figures below 40 m have shown an interestingly steady rise after the thermocline was established. Some aluminium determinations during the period gave figures from 0·2 to 2·0 μg atom Al/l. with no obvious relationship to the silicon concentrations. Some earlier (unpublished) analyses of Western Atlantic water showed values in excess of 10 μg atom Al/l. in water below 1000 m.

The series of experiments on biological differences between sea waters was continued by Dr D. P. Wilson and Mr F. A. J. Armstrong during the spring of 1954. Unfortunately weather conditions much interfered with the collection of the water samples, and on several occasions experiments could not be carried out as planned. None the less, some preliminary results indicated that there is a marked difference between bottom and surface waters from the same hydrographical position, the eggs and larvae of *Echinus esculentus* developing more normally in one than in the other. However, in view of the fact that these experiments cannot be completed and confirmed until another year, a description of what has been done might ultimately prove to be misleading. In addition, some further attempts were made to free water samples from bacteria at the time of collection. Few of these attempts were completely successful, but those that were gave little support to the hypothesis that the major differences which have been observed can be accounted for by changes brought about by the multiplication of bacteria in the water samples in the period between collection and testing.

No noticeable change in the poor macroplankton production level association with the prevailing *Sagitta setosa* conditions has been observed by Mr P. G. Corbin during 1954.

During her tenure of a British Council Scholarship, Señora A. Alvariño, biologist on the staff of the Spanish Institute of Oceanography, assisted

Mr Corbin in the analysis of the routine weekly macroplankton samples taken during 1950–53 in half-hour hauls of the 2 m stramin ring trawl off the Eddystone. It is intended to publish these observations in one paper.

In September 1954 Mr Corbin had an opportunity aboard R.R.S. *Discovery II* of carrying out some preliminary trials of the 15 ft, self-depressing mid-water trawl referred to in the last Report of Council. It was evident from the lie of the net and its stability when towing that the principle of design is sound, but to attain a good fishing depth when towed at some speed without paying out a disproportionately long length of warp will entail certain modifications.

Mr Corbin gave some assistance to Señora A. Alvariño in her plankton work and accompanied her on the May 1954 cruise in R.V. *Sarsia*. This cruise extended southwards from Ushant along the French Biscayan continental shelf to the latitude of Belle Ile. Señora Alvariño's analysis of the collections taken revealed some interesting new records of *Sagitta frederici*, a species not previously taken in the Atlantic farther north than the French Moroccan shelf.

The collection of species-pure cultures of marine phytoplankton organisms has been maintained by Dr Mary Parke throughout the year, and a number of new forms have been added to the collection. During the year cultures for research purposes have been sent abroad and have also been produced for the use of research workers in the laboratory.

Work is continuing on new members of the Chrysophyceae. These new organisms and also forms already described are being studied by means of the electron microscope, and Prof. Irene Manton is collaborating with Dr Parke in this part of the investigation.

Miss D. Ballantine has continued work on the two species of *Gymnodinium* in the culture collection, and it is hoped that descriptions of them will shortly be published.

One of these species (*G. veneficum* nom. prov.) has been shown to be highly toxic to fish and to some other marine animals, notably molluscs, and the physiological effect of the toxin on whole animals has been studied. Dr Abbott, Dr Carlisle and Miss Ballantine have been checking the effect of the toxin on isolated preparations of nerves and muscles and on perfused hearts, in an attempt to learn something of its pharmacology and they are also trying to isolate the toxin both from the cells and from the supernatant culture water.

Miss Ballantine and Dr J. E. Morton have been studying the feeding rate and digestive capabilities of the small lamellibranch, *Lasaea rubra*, when fed on a variety of cultures of marine algae. It was found that *Lasaea* was capable of filtering organisms and particles from about 1–40 μ in size, but that digestion of armoured forms (dinoflagellates and diatoms) was very slow, if any.

Macro-Fauna and Flora

Dr D. P. Wilson's experiments on the settlement of the larvae of *Ophelia bicornis*, continued during the summer of 1954, indicated very clearly that the presence of living micro-organisms on the sand grains is a primary factor in inducing larvae to settle and metamorphose. It appears that these organisms, perhaps mainly bacteria, must be neither too few nor too abundant to make an otherwise clean sand fully attractive to the larvae. It is this coating of living organisms which constitutes the food of the adult worms, which swallow the sand grains. On the other hand, dead organic matter is unattractive to the larvae and may be repellent. It is possible that not only the relative abundance of the micro-organisms present influences the larvae testing the sand grains, but that different kinds are more attractive than others. So far very few experiments to determine this aspect of the problem have been possible. A paper describing the latest results will be published in a future number of the *Journal*.

Dr G. A. Steven's routine studies of the spring mackerel fishery at Newlyn, Cornwall, have been continued. The moderate success achieved by that fishery in the spring of this year was in good agreement with his forecast. The outlook for next year is that only moderately successful fishing is again to be expected.

The trawling surveys mentioned in last year's report have been continued. No new trends of particular significance have become evident.

The experiments with artificial bait for crab and lobster pots have now reached a stage when small-scale field experiments are called for. Several members of the trade have offered to collaborate in such experiments, and preparations for them are now well in hand.

In carrying out an echo-sounder survey in an area known as La Chapelle Bank, about 100 miles west of the north-west corner of France, Dr Steven obtained echo traces showing that the sea floor in the vicinity of the 100 fm contour is thrown into a remarkably uniform series of undulations running parallel with the 100 fm contour which marks the beginning of the continental slope in that area. Bottom sampling has confirmed that these undulations are sand waves which extend eastwards (=inwards) from the continental edge for some 10 miles. The 'waves' are of very uniform height—about 35 ft from crest to trough—and roughly half a mile from crest to crest. It seems probable that these sand waves reflect the energy pattern of waves in the overlying water. This deserves further investigation.

Mr G. M. Spooner has continued the faunistic work on isopods and amphipods referred to previously, the results of which will be largely incorporated in the revised fauna list of these groups. Amongst material derived from Mr G. R. Forster's hand-collected rocks and weeds, that from the southern reef of the Eddystone is noteworthy. The fauna of the tufted weeds growing here on exposed rocks intertidally is dominated by *Parajassa pelagica* and *Idothea pelagica*, accompanied by *Hyale pontica*, *H. perrieri*, and, a species new to the British fauna, *Stenothoë spinimana*. This last was previously known from Brittany (rarely) to W. Africa.

Attention has been given by Mr Spooner to insects inhabiting the intertidal zone, in particular the Diptera. Ecologically these fall into two distinct groups. (*a*) Flies that breed in seaweed litter accumulated in the H.W. zones, or at a somewhat lower level in gullies. These include one small, but numerous, sphaerocerid (*Thoracochaeta zosterae*), four coelopids or 'Kelp flies', and five small aberrant empidids predatory on the others. (*b*) A more varied class whose larvae live amongst algae or in rock crevices, etc., in the intertidal zone, the adults of the more active species ranging over the whole tidal area when uncovered. The predatory cordylurids are represented by *Ceratinostoma ostiorum*; and the muscids by the abundant *Fucellia maritima*, also predatory to some degree as an adult. The Aphrosylines are a distinct marine subfamily of the Dolichopodidae, represented in this area by the larger *Aphrosylus celtiber* and *A. raptor*, and the smaller *A. ferox* and *A. mitis*, the first and third being generally common on the rocky shore. Two separable dolichopodid larvae have been recognized from algal tufts in the mid-tide zone, and these are expected to belong to *celtiber* and *ferox*. *Aphrosylus*, too, are predatory in the adult stage, and like *Ceratinostoma* and *Fucellia*, have a predominantly grey colour, though this is quite atypical for the family. Nematocerous families represented are Tipulidae and Chironomidae. Larvae of the tipulid *Geranomyia unicolor* are now well-known inhabitants of the upper half of the tidal zone, sheltering amongst the lichen *Pygmaea pumila* or alga *Catenella repens*; while the adults fly round shaded rocks or damp patches on cliffs in the H.W. region. Five chironomid species regularly occur on the shore in the Plymouth Area; the orthocladines *Hydrobaenus thalassophila*, *Cricotopus fucicola*, and *C. vitripennis*, and the clunionines *Thalassomyia frauenfeldi* and *Clunio marinus*. The larvae are frequent in various intertidal weeds, and an attempt is being made to assign these to their correct adult.

Mr Corbin has continued his observations on the Lucernariidae and on the sucker fish, *Lepadogaster couchii*.

Dr H. G. Vevers has resumed observations, started some years ago, on the animals which live sheltered in clumps of the bryozoans *Cellaria* and *Lepralia*. These clumps occur in the trawling grounds and a measure of their frequency has been obtained from a study of underwater photographs. In particular, the delicate calcareous meshwork of the *Cellaria* colonies form safe nurseries for the young of many benthonic invertebrates. Among these may be mentioned the regularly occurring populations of young queen scallops (*Chlamys opercularis*)—a cubic foot of *Cellaria* colony may contain 100–200 of these molluscs, with a mean shell length of 60 mm.

Dr Vevers is also working with Miss D. Ballantine on the pigments of certain marine flagellates, and in collaboration with Dr G. Y. Kennedy, of the University of Sheffield, has published a paper in Vol. 33, No. 3, of the *Journal*, on the occurrence of porphyrins in certain marine invertebrates. This work has shown the presence of uroporphyrin I in the integument of two molluscs (one tectibranch and one nudibranch), of coproporphyrin III in the viscera of three polychaetes, and of protoporphyrin in three echinoderms. Of particular interest is the occurrence of free chlorocruoroporphyrin in two of these three echinoderms, the phanerozonian starfishes *Luidia ciliaris* and *Astropecten irregularis*.

Mr N. A. Holme has started taking samples of the bottom fauna at a position near the Eddystone. These will be repeated at regular intervals in an attempt to correlate changes with the hydrography of the area. The first samples, taken in August 1954, are comparable with those previously taken in 1950, but there have been appreciable changes in numbers of a few species, notably an increase in numbers of the lamellibranch *Abra alba*. Sampling will have to be continued over a period of years, however, before the significance of any such changes can be appreciated.

Work has continued on the ecology of lamellibranchs on the sea bed, with particular reference to the Veneridae, some of which are important constituents of the bottom fauna. A number of collections have been made on beaches in Devon and Cornwall, but little work has been possible at sea during 1954.

For taking a small sample of the sea bed an improved form of the Hunt 'suction-sampler' has been made. The instrument is easier to handle, as the glass plate sealing the air-chamber has been eliminated, and a number of other modifications have been made. The sampler is effective to depths of at least 200 m, and seldom fails to take a sample.

Observations on the opisthobranch mollusc *Akera bullata* have been made, and notes on the ecology and swimming movements of this species are to be published in a joint paper with Dr J. E. Morton in Vol. 34, No. 1, of the *Journal*. Mr Holme has collaborated with Mr Armstrong in the production of a short film to show the swimming movements of this mollusc.

Dr Carlisle has continued his studies of Plymouth tunicates and has contributed papers on this subject to the *Journal*.

Mr G. R. Forster has completed a note on the sublittoral fauna of Stoke Point Reef. As it was found from the 1953 underwater observations that sponges were frequently abundant, a general study has been made of this group to facilitate their identification. One of the commoner sublittoral sponges has been found to be *Hemimycale* (*Stylotella*) *columella*, only once previously recorded from Plymouth.

The diving technique and collecting methods have been improved, and much more material has been obtained for rock fauna studies. In August and September a total of twenty-nine dives was made outside the Sound, with the assistance of Mr R. U. Gooding. This total includes twelve dives made during a fortnight's cruise to Dartmouth and Torbay in Mr Forster's own yacht *Sunset*.

Additions have been made during the year to the Laboratory's museum collection of algal material and many gaps have been filled both in the Herbarium collection and in the preserved collection. The spread of some of the more southern forms which have appeared on the south coast is being followed.

Physiology of Marine Organisms

During his tenure of a Guggenheim Fellowship Dr J. A. C. Nicol spent most of his time at the Scripps Institution of Oceanography, La Jolla, California, where he continued his researches on the nervous control of luminescence. Most of his attention was devoted to a study of luminescence in the pennatulid *Renilla*. Bright luminous waves, very characteristic of this animal, are controlled by a nerve net which shows non-polarized through-conduction and neuro-effector facilitation of a mode very similar to that discovered in neuromuscular responses of other coelenterates. Information was also obtained for recovery of luminescence after light exposure. Two papers dealing with this work have been accepted for publication in the *Journal of Experimental Biology*. Opportunities were also taken to investigate luminescence in sea pens (*Leioptilus*), California singing fish (*Porichthys*), and in hydromedusae, both at the Scripps Institution, and at the Friday Harbor Laboratories. Visits were paid to other campuses of the University of California, and to the Kerckhoff and Hopkins Marine Laboratories. Dr Nicol also attended a conference on luminescence at Pacific Grove, California, the proceedings of which will shortly be published.

Dr J. S. Alexandrowicz has continued his studies on the muscle receptor organs of crustaceans. The structure of these elements appears to follow the same pattern in various groups of crustaceans, but certain peculiar arrangements were observed in isopods and mysids. In *Ligia oceanica* and *Idotea emarginata* there are also receptors with muscles which extend over two segments, and in *Praunus flexuosus* the muscle components of the receptors of three segments can be fused end to end.

Dr Alexandrowicz has also continued his studies on the innervation of the crustacean heart. An account of his investigations of this system in *Marinogammarus marinus* has been published in Vol. 33, No. 3, of the *Journal*. The observations on *Praunus flexuosus* have shown that the Mysidacea also have three systems of heart nerves (as found previously in other crustaceans), i.e. a local system made up of no less than six neurons, the nerves connecting the local system with the central nervous system, and the nerves of the arterial valves. These results have been published in the *Journal*. A peculiar arrangement of the heart nerves has been observed in *Leander serratus*, in that the nerves coming from the central nervous system enter the ganglionic trunk at its posterior end. The nerve fibres supplying the pericardial muscles have been found to travel by two different ways, some being carried by the segmental nerves, while others are branches of an unpaired nerve emerging on the dorsal surface of the infraoesophageal ganglion.

Dr D. B. Carlisle has continued his work on the endocrinology of moulting in Crustacea. The topography of the endocrine organs of the protocerebrum of Isopoda is similar to that of Natantia, and in the hormonal control of moulting this group appears to follow the same pattern. Eyestalk ablation performed on our native stock of *Leander serratus* led to slower moulting, whereas in *L. serratus* collected at Roscoff the opposite was true, as previously reported by P. Drach. These results are taken as supporting the hypothesis that the eyestalk secretes both a moult-inhibiting and a moult-accelerating hormone.

In collaboration with Mme M. Dupont-Raabe and Sir Francis Knowles, Dr Carlisle has begun an investigation into the separation and characterization of chromactivating substances in prawns and insects. Some measure of success has attended this attempt.

Dr B. C. Abbott has continued measurements on the uptake by marine organisms of uranium fission products—using in particular yttrium, ruthenium and niobium. He and Dr Carlisle have started an investigation into the uptake by ascidians of radioactive niobium, an element which Dr Carlisle has recently found to be present in relatively large amounts in some ascidians. Slow accumulation and retention of niobium has been demonstrated in *Molgula* using the isotope ^{95}Nb, but none is retained in *Ciona*. Autoradiographs have confirmed the uptake in *Molgula* and its absence in *Ciona*.

Dr Abbott has used the radioisotope ^{42}K in a study of potassium exchange in green algae, and has estimated the ionic flux across cell membranes at various levels of illumination.

The rate of leaching of iodide from frog and other muscles after previous equilibration with Ringer solution in which iodide replaces a portion of the chloride ion has been measured in conjunction with experiments of Prof. A. V. Hill on the effects of iodide ion on isolated muscle twitches.

Preliminary experiments on the heat production in isolated byssal retractor muscles of *Mytilus* show a very small heat rate in a maintained tetanus. The effect of width of stimulus pulse and repetition frequency on the threshold voltage for isolated *Mytilus* muscle was investigated in collaboration with M. Van de Vyver from Louvain.

Library

The thanks of the Association are again due to many foreign Government Departments, to Universities and to other Institutions at home and abroad for copies of books and current numbers of periodicals either presented to the Library or received in exchange for the *Journal* of the Association.

Thanks are also due to those who have sent books or reprints of their papers, which are much appreciated.

Dr Anna M. Bidder and Mrs Barclay Russell have generously presented to the Library a collection of reprints which belonged to the late Dr G. P. Bidder.

During the year the library of books and reprints presented by Dr Marie V. Lebour has been catalogued and incorporated in the general index.

The Library has again been much used by visiting members of the Association.

Published Memoirs

Volume 33, No. 1, of the *Journal* was published in February 1954, No. 2 in June 1954 and No. 3 in October 1954; and Vol. 34, No. 1 in February 1955.

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Membership of the Association

The total number of members on 31 March 1955 was 827, being 57 more than on 31 March 1954; of these the number of life members was 93 and of annual members 734. The number of associate members is five.

Momber Bequest

Under the terms of the will of the late E. R. T. Momber, who died in 1911, the Marine Biological Association has received a bequest of £1072.

The Council have decided to use this money for the construction of new outside seawater tanks for research purposes.

Grant from Rockefeller Foundation

The thanks of the Council are due to the Trustees of the Rockefeller Foundation for a generous grant of 30,000 dollars for the promotion of research at the Plymouth laboratory. The grant is to be spread over a period of five years as from 1 October 1954, and not more than 7500 dollars are to be spent in any one year.

Finance

General Fund. The thanks of the Council are again due to the Development Commissioners for their continued support of the general work of the laboratory.

Capital Grant. The Council wish to record their thanks to the Development Commissioners for a substantial Capital Grant to meet the cost of extending the library and constructing a new store for dogfish and other large marine specimens.

Private Income. The Council gratefully acknowledges the following generous grants received during the year:

From the Fishmongers' Company (£500), the Royal Society (£50), British Association (£50), Physiological Society (£30), the Cornwall Sea Fisheries Committee (£10), the Universities of London (£210), Cambridge (£125), Oxford (£100), Bristol (£50), Birmingham (£31. 10*s*.), Leeds (£20), Durham (£10. 10*s*.), Manchester (£10. 10*s*.), Sheffield (£10. 10*s*.), Reading (£10. 10*s*.), Nottingham (£10. 10*s*.), Hull (£10. 10*s*.), Exeter (£10. 10*s*.), Leicester (£10. 10*s*.), and the Imperial College of Science and Technology (£10).

President, Vice-Presidents, Officers and Council

The following is the list of those proposed by the Council for election for the year 1955–56:

President

Prof. A. V. Hill, C.H., O.B.E., Sc.D., F.R.S.

Vice-Presidents

The Earl of Iveagh, K.G., C.B., C.M.G.
Sir Nicholas E. Waterhouse, K.B.E.
Col. Sir Edward T. Peel, K.B.E., D.S.O., M.C.
Vice-Admiral Sir John A. Edgell, K.B.E., C.B., F.R.S.
Sir Edward J. Salisbury, Kt., C.B.E., D.Sc., Sec.R.S.
Admiral Sir Aubrey C. H. Smith, K.B.E., C.B., M.V.O.
A. T. A. Dobson, C.B., C.V.O., C.B.E.
Major E. G. Christie-Miller
Morley H. Neale, C.B.E.
The Earl of Verulam
Prof. Sir James Gray, Kt., C.B.E., M.C., Sc.D., LL.D., F.R.S.

COUNCIL

To retire in 1956

J. N. Carruthers, D.Sc.
Prof. H. Munro Fox, F.R.S.
Prof. A. L. Hodgkin, F.R.S.
Prof. J. E. Smith, Sc.D.
Prof. V. C. Wynne-Edwards

To retire in 1957

Miss Anna M. Bidder, Ph.D.
D. J. Crisp, Ph.D.
Prof. J. E. Harris, Ph.D.
C. E. Lucas, D.Sc.
Prof. C. M. Yonge, C.B.E., D.Sc., F.R.S.

To retire in 1958

Miss Vera Fretter, D.Sc.
Michael Graham, C.M.G., O.B.E.
N. A. Mackintosh, C.B.E., D.Sc.
G. E. Newell, Ph.D.
Prof. Lily Newton, D.Sc.

Hon. Treasurer

Major E. G. Christie-Miller, 38 Hyde Park Street, London, W. 2.

Secretary

F. S. Russell, C.B.E., D.S.C., D.F.C., F.R.S.
The Laboratory, Citadel Hill, Plymouth

The following Governors are also members of the Council:

R. G. R. Wall (Ministry of Agriculture and Fisheries)
The Worshipful Company of Fishmongers:
  The Prime Warden
  Major E. G. Christie-Miller
  Harrison S. Edwards
Prof. A. C. Hardy, D.Sc., F.R.S. (Oxford University)
S. Smith, Ph.D. (Cambridge University)
Edward Hindle, Sc.D., F.R.S. (British Association)
H. W. Parker, D.Sc. (Zoological Society)
Prof. Sir James Gray, Kt., C.B.E., M.C., Sc.D., LL.D., F.R.S. (Royal Society)

BALANCE SHEET 1954–55

THE MARINE BIOLOGICAL ASSOCIATION OF THE UNITED KINGDOM

BALANCE SHEET 31 March 1955

	£	£
Capital Reserve Account:		
As at 31st March 1954	170,447	
Add: Expenditure on fixed assets recovered	525	
		170,972
Surplus Account:		
As at 31st March 1954	11,452	
Less: Excess of expenditure over income for the year	3,306	
		8,146
		179,118
Balances on Special Funds		10,214
Current Liabilities:		
Sundry Creditors and Accrued Expenses	2,137	
Subscriptions and Grants received in advance	146	
Equipment and Apparatus—R.V. 'Sarsia'	838	
		3,121
		£192,453

Note: Capital commitments outstanding amount to £8,522 (1954 £18,072) in respect of the Library Extension and Dogfish House of which £6,815 will be recoverable under a Development Fund Grant.

	£	£
Fixed Assets:		
At valuations as estimated by the Director at 31st March 1955:		
Boats and Equipment:		
M.F.V. 'Sula'	11,000	
R.L. 'Gammarus'	200	
	11,200	
Laboratory Apparatus, Equipment and Machinery at cost	11,060	
Library at valuation of Mr Ridgill Trout in January 1941 plus additions at cost	20,900	
R.V. 'Sarsia' at cost	137,761	
		180,921
Investments:		
General Fund, at cost	232	
(Market value £217; last year £228)		
Composition Fees Fund, at cost	1,219	
(Market value £1,110; last year £1,013)		
E. T. Browne Bequest Funds at cost	4,013	
(Market value £3,440; last year £3,441)		
		5,464
Current Assets:		
Stocks on Hand as valued by the Director	1,550	
Sundry Debtors	791	
Prepayments	135	
Balance at Bank and Cash in Hand	3,592	
		6,068
		£192,453

John E. Harris } *Members of the Council*
O. Lowenstein }

Report of the Auditors to the Members of the Marine Biological Association of the United Kingdom:

Capital expenditure on the erection of buildings on land held on lease from the War Department is excluded. Subject to the foregoing, in our opinion and to the best of our information and according to the explanations given to us, the above balance sheet and annexed income and expenditure account give a true and fair view of the state of the Association's affairs as at 31st March 1955, and of the excess of expenditure over income for the year ended on that date.

We have obtained all the information and explanations which to the best of our knowledge and belief were necessary for our audit. In our opinion the Association has kept proper books of account and the above mentioned accounts, which are in agreement therewith, give in the prescribed manner the information required by the Companies Act, 1948.

Norwich Union House
2 St Andrew's Cross
Plymouth
20 *May* 1955

Price Waterhouse & Co
Chartered Accountants

INCOME AND EXPENDITURE ACCOUNT FOR THE YEAR ENDED 31ST MARCH 1955

	£	£
SALARIES, NATIONAL INSURANCE AND SUPERANNUATION SCHEME CONTRIBUTIONS		27,537
LABORATORY AND BOATS' CREWS' WAGES, NATIONAL INSURANCE, SUPERANNUATION SCHEME CONTRIBUTIONS AND EMPLOYER'S LIABILITY INSURANCE		21,643
UPKEEP OF LIBRARY		651
SCIENTIFIC PUBLICATIONS, *less* Sales		1,531
UPKEEP OF LABORATORIES:		
Buildings and machinery	434	
Electricity, Gas, Coal and Water	1,351	
Chemicals and Apparatus	1,127	
Rents and Insurances	161	
Travelling Expenses...	711	
Audit Fees (including £63 in respect of previous year)	189	
Stationery, Postage, Telephone and Sundries	888	
Specimens	202	
		5,063
MAINTENANCE AND HIRE OF BOATS:		
Petrol, Oil, Paraffin, etc.	1,298	
Maintenance and Repairs	4,680	
Collecting Expenses and Upkeep of Truck	293	
Insurances	2,472	
Hire of Decca Navigator—R.V. 'Sarsia'	395	
Hire of R.V. 'Sabella'	—	
		9,138
ENTERTAINMENT EXPENSES		42
BALANCE being excess of income over expenditure for the year ...		—
		£65,605

Note: No depreciation is provided on fixed assets.

	£	£	£
GRANTS AND TABLE RENTS:			
Ministry of Agriculture, Fisheries and Food Grant from Development Fund		55,866	
Fishmongers' Company		400	
Miscellaneous (including British Association £50, Royal Society £50, Physiological Society £30, Cornwall Sea Fisheries Committee £10, Universities of London £210, Cambridge £125, Oxford £100, Bristol £50, Birmingham £31. 10*s*., Leeds £20, Durham £10. 10*s*., Exeter £10. 10*s*., Leicester £10. 10*s*., Manchester £10. 10*s*., Nottingham £10. 10*s*., Hull £10. 10*s*., Reading £10. 10*s*. and Sheffield £10. 10*s*., Imperial College £10, Ministry of Works £104, Imperial Chemical Industries Ltd £52. 10*s*. and International Paints Ltd. £39. 7*s*. 6*d*.) ...		1,233	
			57,499
SUBSCRIPTIONS (excluding those received in advance)			699
SALES:			
Specimens		2,220	
Fish		358	
	£		
Nets, Gear and Hydrographical Apparatus	439		
Less: Cost of Materials	400		
		39	
			2,617
INTEREST ON INVESTMENTS			44
INTEREST ON BANK DEPOSITS, *less* Charges			39
SALE OF DR M. V. LEBOUR'S BOOK			5
AQUARIUM:			
Admission Fees		1,655	
Sale of Guides		116	
		1,771	
Less: Maintenance, Printing and Advertising		375	
			1,396
BALANCE being excess of expenditure over income for the year ...			3,306
			£65,605

MOVEMENTS ON SPECIAL FUNDS DURING THE YEAR TO 31ST MARCH 1955

	Aquarium Sinking Fund	Composition Fees	E. T. Browne Bequest			A. R. T. Momber Bequest	Rockefeller Foundation	War Damage Compensation Part II	Buildings Reconstruction	Library Extension and Dogfish House	Research Funds*	TOTAL
			Library	Special Apparatus	Scientific Publications							
	£	£	£	£	£	£	£	£	£	£	£	£
BALANCE AT 31ST MARCH 1954	307	1,093	1,296	2,795	(21)	942	—	957	1,136	—	760	9,265
Add: Receipts during year:												
Grants	—	—	—	—	—	—	1,343	—	—	9,550	1,956	12,849
Income from investments	—	—	39	81	—	—	—	—	—	—	—	120
Bank deposit interest	4	—	—	—	—	14	—	—	—	—	—	18
Other receipts	30	158	—	—	—	134	—	—	—	—	—	322
Inter-Fund transfers	—	—	—	—	—	—	—	(564)	(1,136)	1,700	—	—
	341	1,251	1,335	2,876	(21)	1,090	1,343	393	—	11,250	2,716	22,574
Deduct: Expenditure	—	—	—	—	—	—	825	348	—	9,683	1,504	12,360
BALANCE AT 31ST MARCH 1955	£341	£1,251	£1,335	£2,876	£(21)	£1,090	£518	£45	—	£1,567	£1,212	£10,214

* Including International Paints Research Fellowship.

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